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Chromosome Aberrations in Peripheral Lymphocytes of College Students as a Response to Photochemical Air Pollution

Loma Linda Univ., CA

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This research project was designed to confirm and extend to a more homogeneous group the observations of Scott and Burkart (9) in their study of chromosome aberrations in the peripheral lymphocytes of students attending the University of Southern California. The primary objective of this study was to compare whether the incidence of chromosome aberrations in periphera; lymphocytes of young adults emigrating from and imigrating to an area of low levels differed significantly from students indigenous to the respective aeras.

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CHROMOSOME ABERRATIONS IN PERIPHERAL LYMPHOCYTES OF COLLEGE STUDENTS AS A RESPONSE TO PHOTOCHEMICAL AIR POLLUTION

by

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28 February 1980

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The study described in this report and a previously reported pilot study (EPA 600/1-78-054) were undertaken to evaluate possible mutagenicity of daily exposure to ozone concentrations in ambient air. Both studies offered EPA's Health Effects Research Laboratory a new biological indicator of community air pollution exposure, the examination of circulating lymphocytes for chromosome abnormalities. The results of both studies are essentially negative, from which it can be concluded that cytogenetic analysis of peripheral lymphocytes is of little value in detecting effects of photochemical air pollution exposure.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

This research project was designed to confirm and extend to a more homogeneous group the observations of Scott and Burkart (9) in their study of chromosome aberrations in the peripheral lymphocytes of students attending the University of Southern California. The primary objective of this study was to compare whether the incidence of chromosome aberrations in peripheral lymphocytes of young adults emigrating from and immigrating to an area with perpetually high levels of photochemical air pollutants versus an area of low levels differed significantly from students indigenous to the respective areas.

Two hundred nine (209) male and female first-year students at the La Sierra campus of Loma Linda University (high smog) and two hundred six (206) male and female first-year students at Pacific Union College (low smog) were pre-enrolled after completing a lifestyle questionnaire or responding to a similar telephone-administered questionnaire. They were assigned to groups according to their previous life-time exposure to air pollution and the college in which they were matriculating. This resulted in 4 groups: low smog to low smog, low smog to high smog, high smog to low smog, and high smog to high smog.

Blood samples and updated health histories were obtained from the students at the following times: September-October 1976 (410 students); November 1976 (407 students); April 1977 (369 students); and September-October 1977 (317 students). All blood samples were cultured and slides prepared by the Genetics Research and Chromosome Services, Loma Linda University Medical Center. One hundred cells for each student were analyzed for chromosome and chromatid aberrations at Utah Biomedical Test Laboratory.

A careful analysis of the data does not support the hypothesis that living (or attending school) in the South Coast Air Basin of southern California, with its high photochemical air pollution, significantly affects the integrity of peripheral blood lymphocyte chromosomes. Nor is there evidence that those living in areas of little or no photochemical air pollution have statistically significantly fewer chromosome abnormalities.

This study was carried out by Loma Linda University and funded by the United States Environmental Protection Agency, grant number 804606-02.

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INTRODUCTION

SUMMARY BACKGROUND

Conflicting evidence continues to accumulate that exposure of human, animal and plant tissues to the constituents of photochemical air pollution may result in increased chromosome aberrations. Of particular concern is the potential danger that exposure of humans to presently existing ambient levels of community photochemical smog may result in mutagenic changes leading to congenital anomalies. Considerable evidence exists for the induction of both point and chromosome aberrations in several species. Fetner (1) observed that chromosome breakage and aberrations were present in *Vicia faba* root meristem cells after exposure to 0.4 ppm ozone. Both qualitative and quantitative increases in centric and dicentric ring aberrations occurred with increasing exposure (1). Sachsenamier, et al (2) found ozone-induced chromosome aberrations in chick embryo fibroblasts. Chinese hamsters, exposed to 0.2 parts per million ozone for five hours, were observed by Zelac (3) to have an increase in chromosome aberrations in their peripheral blood lymphocytes. However, Gooch, et al (4) found no significant level of chromosome damage in similarly exposed Chinese hamsters. This difference may have resulted from Gooch, et al observing bone marrow cells instead of circulating lymphocytes. In repeating the experiment of Zelac, et al, Tice, et al (5) observed no increase in the level of any chromosome aberration type.

Studies of ozone effects on human cells have been limited. Fetner (6), in an early *in vitro* study, noted chromosome damage (chromatid deletions) in human cells after exposure to extremely high concentations of ozone (8.0 ppm) for up to 10 minutes. In another *in vitro* study Gooch, et al (4) subjected human peripheral leukocytes to some 23 different combinations of ozone concentration and duration of exposure. Their data (4) indicated no consistent increase in chromosome abnormalities. In addition to these *in vitro* human studies several *in vivo* studies have recently been reported. Merz, et al (7) observed a significant increase in chromatid-type deletions in circulating lymphocytes in humans exposed to 0.5 parts per million ozone for six to ten hours. Only one (of six males) of the subjects exhibited chromosome-type aberrations. In marked contrast to the previous study McKenzie, et al (8) found no evidence of significant chromosome aberrations in the circulating lymphocytes of thirty males exposed to 0.4 parts per million ozone for four hours. They concluded that the data indicated no apparent detectable human cytogenetic effect due to exposure to ozone under the conditions of the experiment. The lower concentration (0.4 ppm versus

0.5 ppm) and the shorter duration (4 hours versus 6 to 10 hours) may explain the differences between the study of McKenzie, et al (8) and Merz, et al (7). In a study of 256 college students exposed to ambient levels of photochemical smog, Scott and Burkart (9) concluded that their data indicated that prolonged exposure to Los Angeles area smog elevated the level of chromosome aberrations. Guerrero, et al (10) found no significant increase in sister chromatid exchange in circulating lymphocytes obtained from humans exposed to 0.5 parts per million ozone for two hours. However, when diploid human lung cells were exposed to ozone *in vitro* there was a clear dose-related increase in sister chromatid exchanges (10). Bloom (11) found no significant increase in the frequency of chromosome aberrations in 273 U.S. Army welder trainees exposed to varying levels of oxidants (ozone up to .015 ppm; NO from 0.2 ppm to 6.0 ppm, and NO_x from 0.5 ppm to 8.0 ppm).

SCOPE OF WORK

To better understand the potential health effects of man's exposure to currently existing ambient levels of photochemical air pollution this investigation was carried out by Loma Linda University under sponsorship of the Environmental Protection Agency. Its primary objective was to determine whether or not exposure to photochemical smog results in aberrations of the chromosomes in peripheral blood lymphocytes of healthy young adults. Four hundred and six first-year male and female students at two colleges--Pacific Union College, Angwin, California and the La Sierra Campus of Loma Linda University, Riverside, California--were enrolled in this two-year study. The Pacific Union College campus is located in an area of low photochemical smog in northern California and the La Sierra campus is located in one of the highest photochemical smog areas of southern California.

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CONCLUSIONS

Although we were unable, because of technical difficulties in blood drawings three and four to complete the statistical analyses of all groups for all four blood sampling periods, it is felt that this study is significant in that four contrasting groups, with respect to exposure to photochemical air pollution in the South Coast Air Basin of southern California, were cytogenetically examined at the conclusion of the high pollution season and again two and six months later.

No statistically significant differences in the aberrations rates for the types of chromosome abnormalities studied were observed between the four exposure groups of students at each of the sampling periods or among the same students over the duration of the study. An exception of statistical significance was the elevated rate of stable changes at the initial blood sampling period for males in the low-to-high group. Although this group did not have a statistically significant elevation in rate when the initial criterion of allowing students to arrive on campus within up to 15 days prior to blood drawing was applied the rate became increasingly elevated over the other groups and was statistically significant when 2 successively more stringent criteria for arrival time were applied, namely within 3 days of blood drawing and then within 1 day. Increased differences, but not as statistically significant were also noted for females in the same group. These elevated rates dropped off by the second blood sampling 2 months later.

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RECOMMENDATIONS

It does not appear that the cytogenetic analysis of circulating lymphocytes is the most sensitive indicator of the human health effects of photochemical air pollution. That is, alterations in chemical balances, membrane sensitivity, and immunological responses, etc. likely precede observable morphological changes. In addition, normal distribution curves potentially result in large inter-person variability. Observer selection and interpretation when scoring, results in large intra-student variability and discrepancies between persons who score the same slides. Also, there are many other environmental chemicals and physical forces that affect chromosomes which often cannot be controlled, and therefore confound the study.

Future studies can reduce the intra-student variability by scoring a minimum of 200 metaphases. Study group size could be reduced by using more stringent exclusion criteria such as those used for statistical analyses in this study.

An on-going validity study to check accuracy in chromosome scoring should be carried out at an independent laboratory paralleling any cytogenetic study.

A carefully controlled laboratory study exposing students having no previous exposure to significant levels of ozone could be carried out to test the "shock effect" hypothesis.

CHAPTER 1

MATERIALS AND METHODS

RESEARCH DESIGN

Since it is likely that variables other than smog per se may result in chromosome abnormalities, this study was especially designed to remove such factors. Two college groups were studied--one (La Sierra campus) in the South Coast Air Basin with the resulting high exposure to photochemical smog and one (Pacific Union College) outside the South Coast Air Basin in an area relatively free of air pollution. At each college campus 2 groups of students were enrolled in the study. One group had prior residence histories involving exposure to high levels of photochemical smog and one group had prior residence histories which involved no known high exposures to photochemical smog.

A cross classification of the campus at which students matriculated, with their past residence history, yields the four main subgroups shown in Table 1, page 19. Attempts were made to obtain equal numbers of males and females in each of the major subgroups.

Another aspect of the research design was to enable the study of possible time trends in the rates of chromosome aberrations in students. To accomplish this, blood samples were drawn from students during four periods over a twelve-month period. The first blood drawing was in September when students first arrived on campus and the time of year which normally coincides with the high photochemical smog period in the South Coast Air Basin. The second blood drawing was in November which is generally near the beginning of the lower ozone levels experienced in the South Coast Air Basin beginning in late fall and continuing through the winter months. The third blood drawing was in April, near the end of the lower ozone levels generally experienced in the South Coast Air Basin during the preceding winter months, and the fourth blood drawing was in September when students returned to their respective campuses for another school year. The research design of Table 1, coupled with time sequence of blood drawings, would yield the hypothetical profiles for chromosome abnormality rates shown in Figure 1, page 20, if aberration rates were to lag behind air pollution exposure effects by not more than a few weeks. The blood drawings were scheduled to correspond to inflection points in these expected profiles. Four additional exposure groups are obtained depending on whether students spend the summer prior to the last blood drawing in an area of low or high smog. This study design provides a baseline control group, Group I (Profile A), which should have the same chromosome aberration rate throughout the study. Group III, High to Low (Profile E), enables the observation of how long chromosome aberrations which may result from exposure to photochemical air pollution persist when the student is in a relatively smog free environment. The remaining groups allow the study of the effects of varying levels of photochemical air pollution exposure throughout the course of the year of the study (Profiles B, C, D and F)

SELECTION AND CLASSIFICATION OF STUDENTS FOR STUDY DESIGN GROUPS

Selection Of Students

Lists of names and addresses of all students coming to college for the first time were obtained from each college in July. Seniors were excluded from these lists since they would not be at the college for the final blood drawing in the Fall of 1977. These mailing lists were updated on September 8 and 9 to obtain students coming in during the month of August. Students coming in during the month of August were not included in the low-to-low group as sufficient numbers were already obtained. To ascertain whether students were eligible for the study, would be willing to participate, and to which study design group they belonged, students were mailed an initial screening questionnaire. (See Exhibit 1 of Appendix A.)

The initial mailing went out on August 9, 1976. Approximately two weeks later on August 23, 1976, telephone interviewing to nonrespondents was begun. Interviewers were given an instruction sheet, (see Exhibit 2 of Appendix A) and attended a group training session. In conducting the telephone interviews, they used a telephone version of the screening questionnaire (See Exhibit 3, Appendix A). Each screening questionnaire was accompanied with a cover letter explaining the purpose of the study and requesting the student's cooperation (See Exhibits 4 and 5, Appendix A). It was felt that students would be reluctant in providing individual answers to the questions on cigarette usage and psychedelic drug usage on the telephone, since smoking cigarettes and using psychedelic drugs are forbidden at SDA colleges. To overcome this reluctance and any possible bias, these questions were grouped with two other non-sensitive questions in Section B and individuals were asked only to say if their answer was "yes" to any of the questions. In this way they did not have to indicate to which of the questions their answer was "yes."

To save telephone expense, the telephone interviewer was instructed to terminate the interview at the end of page 1 if the student answered "yes" to any of the questions in Section B. Age and year in college were tacked on to the end of Section B to make it less obvious that the interview was being abruptly terminated. Interviewers were instructed that if the student wished to mail in his questionnaire rather than completing it by telephone, a mailed questionnaire would be acceptable. Three hundred eighty-eight (388) telephone interviews were completed on the phone. Sixty (60) refused to participate. At the time telephone interviewing was begun, the response rate on the mailed questionnaire was 23%. One thousand nine hundred nine (1909) questionnaires were initially mailed out and 432 had been returned by this time with 155 returning refusal cards. A refusal card was included with the cover letter and screening questionnaires (See Exhibit 6, Appendix A). This was done to avoid repeated contacts with students who did not wish to participate and to provide an easy way out for those who might be involved in drug usage but who did not want to say so in view of the college restrictions. After questionnaires had been mailed in or telephone interviews had been completed, students were first screened to ascertain whether they met the critera for inclusion in the study.

Students who answered "yes" to any of questions B 1-4, Section B of the telephone questionnaire, or the equivalent questions of the mailed questionnaire, were excluded from the study. Students who answered "yes" to questions 1-4 of Section A were included or excluded from the study depending on their chemical or drug usage. Students who answered "yes" were screened on an individual basis to ascertain whether the types of chemicals or drugs were serious enough to warrant exclusion from the study. Some medication usage and chemical exposure in questions 1-4 was allowed in groups 2, 3, and 4. In the low-to-low group, no chemical or drug usage was allowed unless it was very slight, as this was the baseline control group and also sufficient students were obtained for this group. This could represent a differential bias for group 1. To cancel out any such possible bias, analyses were repeated excluding all students with covariate exposure from groups 2, 3, and 4 as well as group 1. The age was restricted to 17-22; the year in college, freshman through junior. Only students who were planning to attend the campus during the two years of the study were included, with the exception of some students who indicated that they would be at the Glendale campus to complete the nursing program. These students, however, if freshmen, would be at PUC for the fall quarter and thus, for the September blood drawing and so were allowed. Both village and dormitory students were included.

This questionnaire was pre-tested on different groups of young people of similar age to those students who would be selected and was subsequently refined on the basis of this pre-test and suggestions made by a physician experienced in interviewing and treating individuals with drug or alcohol dependent behaviors.

Procedure For Classifying Cities Inside Of California As To Low Or High Oxidant Levels

At the time of admitting students into the study in the summer of 1976, it was necessary to be able to classify the cities of California from which students came as to high or low smog so that the number of students in the design subgroups of the study could be balanced. Since 1976 air pollution data was not available at that time, and since it was felt that total cumulative exposure in excess of a threshold would be the most meaningful statistic to use in classifying cities, the most recent available cumulative excess oxidant dosage statistics were used. This was a computer listing which was obtained from the State-wide Air Pollution Lab at Riverside, California based on 1971 California Air Resource Board statistics. This listing gave for all of the Air Resources Board Monitoring Stations in California the number of parts per hundred million hours in excess of .08

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ppm oxidant level during 1971. The following criteria for classifying cities in California was used: Less than 500 parts per hundred million hours excess dosage was classififed as low, 500 to 3000 was classified as intermediate, and over 3000 was classified as high. All intermediate cities were given an intermediate code in a separate portion of the data base so that they could be identified if necessary. Attempts were made to exclude students coming from the intermediate regions so that greater contrasts could be seen in this study. A few students from these regions were inadvertently included in the study, however.

For purposes of the study, those few students coming from intermediate regious were classified as coming from either low or high smog areas depending on the proximity of their home city to the South Coast Air Basin and the likelihood of their commuting into the South Coast Air Basin for work or study purposes (see Table 2, page 21). In order for a student to be classified as coming from a high smog area, his home must have been in or adjacent to the South Coast Air Basin. Also taken into consideration was whether the dosage level of the intermediate city tended to be toward the high or low end of the intermediate range.

Procedure For Classifying Cities Outside Of California As To Low Oxidant Levels Or High Oxidant Levels In Relationship To California

Although no students coming from outside of California were classified as high smog, the oxidant levels of their home cities were checked to determine whether the students could be classified as coming from a low oxidant area. The procedure outline by Gerald Nehls, Chief Data Management Staff of EPA in his memo of September 8, 1978 to Dr. Dorothy Calafiore was followed. The first step was to go through section D of the "Air Quality Data 1976 Annual Statistics", EPA publication 450/2-78-009 March, 1978, to classify all cities as to high or low. Based on the California data a criterion for high was defined as greater than or equal to 60 observations above the one hour ozone standard (160 micrograms per cubic meter). This decision was made by comparing the Napa data with the South Coast Air Basin data and looking at other data for areas known to be low in California. This roughly corresponds to a maximum value for ozone of 300 micrograms per cubic meter. Using this criterion all of the cities that are listed for all of the states on this computer output were classified. Ten of the California cities from which students came exceeded the 300 micrograms per cubic meter maximum value criteria, but were below the 3000 parts per hundred million hours above .08 ppm criteria, used for classifying cities in California. Table 2 shows the excess dosage and maximum values for these 10 cities and the way they were classified for the study. All of these cities are outside the South Coast Air Basin. If a city in this study was not on the computer ozone list, then we checked the NO_2 data to see if that city has NO_2 data. Comparing the cities of Napa with the cities of Rubidoux and Riverside on NO_2 data, it appeared that a threshold for "high" of a maximum one-hour value for the year of 300 micrograms per cubic meter would also apply. Another procedure for areas suspected to be low was to look at the total output for the whole area given in the "1973 National Emissions Report", EPA publication 450/2-76-007 May 1976, in Northern California. We were able to classify the entire state of Oregon, for example, as being low on this

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basis. Using this criterion, Colorado was also classified as low except for metropolitan Denver. Cities classified as low on the basis of ozone data were double checked to be sure they were also low for SO_2 data. The definition for low for SO_2 data was taken to be less than 100 micrograms per cubic meter for a 24-hour maximum, using any of the various methods published in the EPA "Air Quality Data 1976 Annual Statistics". The following cities were classified as high SO_2 on this basis -- Dayton, Ohio; Denver, Colorado; El Paso, Texas; New York City; and Salt Lake City, Utah. These low oxidant, high SO_2 cities were given a separate code in the data base so they could be identified. Long Beach, California had a maximum 24-hour SO_2 average of 106 micrograms per cubic meter but was coded as intermediate ozone rather than high SO_2 because of its proximity to the South Coast Air Basin. The classification of cities is shown in Appendix B. It is realized that this is a crude classification in view of the fact that statistics from different sources and with differing measurement techniques were used but this was the best that could be done with the available data. No cities outside of the South Coast Air Basin were classified as High Smog. Thus the classification criteria were used to be sure that cities outside the South Coast Air Basin were low in ozone relative to South Coast Air Basin and to determine which cities in the South Coast Air Basin were low in ozone levels.

Classification Of Students By Study Design Groups

Students were classified by the four design groups of the study according to their past residence and the college at which they were enrolling. Some students between the close of June of 1976 and enrollment in the fall of 1976 had intervening periods different from their past residence history or place of enrollment during the summer. This necessitated an additional four groups into which students were classified according to whether they had an intervening exposure of two or more weeks. The groups were defined as follows:

1. Low smog to low smog
2. Low smog to high smog
3. High smog to low smog
4. High smog to high smog
5. Group 1 with intervening high smog
6. Group 2 with intervening high smog
7. Group 3 with intervening low smog
8. Group 4 with intervening low smog

The minimum length of time for intervening exposure was two weeks, and the maximum was 13 weeks, being the length of the summer recess. Students must have lived at least six months in one place in order to qualify for this study. The total number of intervening high smog weeks in the cases of groups 5 and 6 or the total number of intervening low smog exposure weeks in the cases of groups 7 and 8 was coded on the computer as well as the number of weeks prior to blood drawing at which the last intervening period occurred.

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Another problem which confounded the design somewhat was that some students arrived on campus early. Whenever possible, special blood drawings were made to obtain blood samples from these students within 48 hours of arrival on campus. In a few cases however, we were unable to do this. The number of days prior to blood drawing at which a student arrived on campus was coded so that it could be considered in the statistical analysis.

MEASUREMENTS OF AIR POLLUTANTS AT THE TWO CAMPUSES

To obtain measurements of ambient concentrations of photochemical and other air pollutants, air monitoring equipment was located on the campuses of the two colleges. The CHAMP mobile stations, operated for the EPA by Xonics, Inc., were positioned near the geographic and activity center of each campus to reflect the average exposure to the students during the usual college program.

Each CHAMP Monitoring station had the capability of obtaining continuous ambient readings of the following: ozone, oxides of nitrogen, sulfur dioxide, relative humidity, ambient temperature, wind direction, wind speed, barometric pressure, total suspended particulates/mass, total suspended particulates/suspended sulfates, total suspended particulates/suspended nitrates, total suspended particulates/Benzo (a) pyrene, respirable suspended particulates/mass, respirable suspended particulates/suspended sulfates, respirable suspended particulates/suspended nitrates, bubbler SO_2, bubbler NO_2.

CHAMP station #0823 was located on the La Sierra campus of Loma Linda University near College Hall at an altitude of 256 meters MSL. The air inlets to the sampling equipment in the station were approximately 3.5 meters from the ground.

CHAMP station #0812 was located on the Pacific Union College campus on a parking lot behind the library at an altitude of 553 meters MSL. The air intake probe was approximately 3.5 meters above ground.

The methods employed in measuring air pollutants and meteorological variables in the CHAMP stations are as follows:*

1. O_3: Ozone Analyzer Bendix Model 8202

   This instrument utilizes the photometric detection of the chemiluminescent reaction produced when ozone and ethylene are combined. The light produced during the reaction is quantified by the photomultiplier tube whose output is amplified, averaged, and displayed. The level of ozone was continuously recorded by computer.

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*Adapted from Xonics, Inc. CHAMP O & M Manual 582A7111/582A7116

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2. NO_x: Total Oxides of Nitrogen Bendix Model 8101

This instrument utilizes the photometric detection of the chemiluminescent reaction produced when ozone and nitric oxide are combined. This technique measures only the nitric oxide portion. Other oxides must be converted from their natural state to nitric oxide before they were monitored.

The analyzer measures nitric oxide (NO), nitrogen dioxide (NO_2), and total oxides (NO_x) as follows:

a. The sample is delivered directly to the detection cell where it is reacted with ozone (which is generated in another portion of the instrument). The light produced by the reaction is quantified by a photomultiplier tube. The output of the detector is amplified and averaged for a period of 30 seconds.

b. At the end of the 30 seconds, the average voltage is transferred to a memory card where it provides an output signal until it is updated one minute later.

c. The NO_x measurement cycle begins as soon as the NO cycle is completed. At this time a three-way solenoid valve diverts the sample through a converter where NO_2 is reduced to NO. The converted sample is now delivered to the detector cell where the measurement is made as in Step (a) and Step (b).

d. The NO_2 concentration is determined by electronically subtracting the NO voltage from the NO_x voltage. This valve is also stored on a memory card which is updated at one minute intervals.

3. SO_2: Total Sulfur Monitor Bendix Model 8300

This instrument utilizes the photometric detection of luminescence produced by sulfur compounds burnt in hydrogen flame. The light produced is in the 394 nanometer wave length region. An optical filter permits the photomultiplier tube to quantify the reaction rate. The output of the photomultiplier tube is exponentially proportional to the concentration of sulfur in the sample; therefore, the output is amplified and linearized before display.

4. Relative Humidity: Yellow Springs Instrument 15132 Dew Point Probe

This instrument is designed to measure dew point temperature from -40°F to +90°F in still air. The miniaturization of the probe allows it to operate with small volumes of air. The probe construction consists of a thermilinear thermister component

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mounted in a housing, a removable bobbin assembly, and protective shield.

The bobbin assembly consists of a spool wound with lithium chloride-impregnated wicking, on which gold bifilar electrodes are wound. The lithium chloride absorbs water from the surrounding air when the vapor pressure of the surrounding air is greater than that of a saturated solution of LiCl. With absorption, the wicking becomes conductive, and heat is generated when voltage is supplied to the electrode. Eventually a heat-moisture equilibrium is reached where the vapor pressure of the small amount of LiCl in solution in the wicking equals the water vapor pressure of the surrounding air. The Thermolinear thermister component senses this temperature. These values are averaged and converted to engineering units in the Data Acquisition System.

5. Ambient Temperature: Xonics Model 310

This system employs a linear Yellow Springs Instrument thermister mounted in a forced air asperated shield. The temperature probe is mounted in the shield which protects it from the weather and eliminates errors due to solar radiation. The probe is mounted on the meterological tower approximately 2 meters above the shelter.

Temperatures from $-20^{\circ}F$ to $120^{\circ}F$ can be monitored. This is converted in the data acquisition system to degrees centigrade.

6. Wind Direction: Xonics Model 510

The wind direction sensor employs a synchro resolver driven by a wind vane. Two 1KH$_2$ signals are generated in the sensor: one is a reference and the other is a signal whose phase is shifted with respect to the reference. Since the phase can shift through 360°, this is proportional to wind direction. A signal conditioner converts the signal to 0 to 5 volts. The data acquisition system takes one grab sample every five minutes.

7. Wind Speed: Xonics Model 510

The windspeed sensor employs a 40 slot perimeter beam chopper driven by a three cup anemometer. A signal frequency proportional to windspeed is produced. This frequency is converted electronically into a 0 to 5 volt signal for 0 to 100 MPH.

8. Barometric Pressure: Yellow Springs Instrument - Barometric Pressure Analog Output Barometer Model 2414

This instrument's sensor, which is in an airtight housing, contains a multicell aneroid sensor which positions the core of a linear variable differential transformer. Its output voltage, which is linear with core position (hence with pressure), is amplified to the level desired. All mechanical and electrical components are designed to achieve linearity and a low temperature coefficient. The aneroid cells are of NISPAN-C and have a thermal expansion coefficient of essentially zero.

9. Total Suspended Particulates and Respirable Suspended Particulates

   The suspended particulates were measured by manual methods as described in EPA Publication 600/1-76-011, January, 1976, "Community Health Environmental Surveillance Studies (CHESS) Air Pollution Monitoring Handbook: Manual Methods."

Continuous readings from all monitoring equipment were recorded within the CHAMP stations. Every five minutes an average of the previous (five minute) period was automatically calculated and electronically transmitted to Research Triangle Park and stored on master tapes at the EPA research facility. Each week the CHAMP station tapes were sent to Research Triangle Park to validate the master tapes.

Table 3, page 22, shows the number of hours of ozone exposure in excess of 0.1 and 0.2 parts per million measured at the PUC and La Sierra stations each month. Table 4, page 23, shows the cumulative ozone exposures in excess of 0.1 ppm and 0.2 ppm at the two monitoring stations prior to the September 1976 blood drawing and between the successive blood drawings.

Monthly averages of daily arithmetic means of the various pollutants and meteorological factors monitored by the CHAMP station on each campus are listed in Table 5, page 24, (ozone, nitrogen dioxide, total hydrocarbons, methane, and particulates), and in Table 6, page 25, temperature and dew point). Sulfur dioxide levels were measured; however, all measurements were below the minimum detectable level of the monitoring equipment (0.02 ppm). It can be seen from table 3 that there are striking differences in ozone exposure at the two campuses when the exposure is measured in terms of hours in excess of 0.1 ppm or 0.2 ppm. In table 5 on the other hand we see that there is a lack of contrasting exposure between the two campuses when daily arithmetic means of ozone levels are compared, although the La Sierra campus is still higher than the Pacific Union College campus except for the winter months December through March. Monthly arithmatic means have the problem of averaging in a large amount of low background levels which are probably insignificant in terms of impact on human health. It was thus felt that hours in excess of a threshold was a much more meaningful measure of pollution exposure.

RATING OF COVARIATES NOT ASSOCIATED WITH OZONE EXPOSURE FOR SEPTEMBER BLOOD DRAWING QUESTIONNAIRE

Each factor measured in the "Adventist College Student Chromosome Study Personal Health History Form" administered to the student at the time of the September 1976 blood drawing was evaluated and rated as to its likelihood of inducing chromosome aberrations. It is recognized that any weighting of the covariates ascertained by questionnaire is accompanied by some risk of misinterpretation and is, therefore, rather arbitrary in nature. However, because of this uncertainty these covariates were used in the statistical analysis only as a dichotomous variable. A rating score of "1" was used for a chemical or exposure suspected of inducing chromosome aberrations where the evidence is inconclusive. A rating score of "2" was used for a chemical or exposure definitely known to induce chromosome abberations. Chemical or other exposures having no known effect on chromosomes were rated as "0". The criteria for "1" and "2" ratings for specific factors are listed below. All factors not listed received "0" ratings.

Questions #3	The only occupational chemicals to which students were exposed which had likelihood of inducing chromosome aberrations were insecticides and pesticides. This was rated as a "1" if it was within thirty days of the blood drawing.
Question #16	Viral infections within thirty days of the blood drawing. The students checked a list of symptoms (see questionnaire). It was felt that the symptom of laryngitis or muscle aches or pains must be present to indicate definite presence of a viral infection. If a student had checked viral infection and at least one of these symptoms, he received a score of "2" for this factor.
Question #17	All students who checked "yes" to having infectious mononucleosis within the last six months were hand checked to be sure that the date they had given was within the last six months. There were a number who had given dates earlier than six months ago. These were discounted. All cases of infectious mononucleosis within the last six months were scored as "2".
Question #22	All students who checked "yes" on question #22 but who did not check "yes" on question #17 were hand-screened to make sure that there was no one who, having failed to mention mononucleosis on question #17, had indicated it on question #22. There were none. The other illnesses which were experienced thirty days or more prior to the blood drawing were scored as zero.
Question #23	Prescription medications. All those who had used prescription medications were hand-screened and graded "1" or "2" according to the type of medication used. The Physician's Desk Reference and the list of clastogen's by Shaw (12) were used in making the ratings.

Question #25	Individuals who had received vaccinations containing live or live attenuated viruses within six weeks of a blood drawing received a score of "2" for this question. All others received a "0" rating. Those vaccinations which were classified as "2" were Rubella (German Measles), Mumps, Polio, Smallpox, and Yellow Fever. The other immunizations which students had received were booster Cholera, DPT, Flu, Gammaglobulin, PPD, TB, Tetanus, and Typhoid. These were all rated zero.
Questions #26 to #28	If a student indicated he had received x-radiation for any of questions 26, 27, or 28, it was rated as "1", if the x-ray had been within one week of the blood drawing, otherwise it was rated zero.
Question #30	If students had frequently or occasionally used cigarettes, cigars or pipes within thirty days of the blood drawing, they received a score of "2".
Question #31	If students had frequently or occasionally experimented with any of the psychedelic drugs within thirty days of the blood drawing, they received a score of "1".

RATING OF COVARIATES NOT ASSOCIATED WITH OZONE EXPOSURE FOR NOVEMBER AND APRIL BLOOD DRAWING QUESTIONNAIRES

Both the November and April blood drawing questionnaires were the same except for minor wording changes. Covariate exposures were rated by the same criteria as the September questionnaire, with the exception of x-rays. The date of x-ray was not included on the November and April questionnaires; thus, the date of exposure in relation to blood drawing is not known. Therefore, x-ray exposure was not coded as a covariate.

BLOOD DRAWING AND HANDLING

Approximately 7 ml of venous blood was withdrawn from each student using sterile technique, into heparinized vacuum test tubes. The tubes were rotated to allow thorough mixing and then kept refrigerated until transported in ice-cooled styrofoam containers to the laboratory later that day. The samples from the La Sierra College campus were transported by car about 25 miles to the laboratory in Loma Linda. The samples from the Pacific Union College campus were transported by a six-passenger twin engine airplane below 7,000 foot altitude about 500 miles to the Redlands, California airport and from there by car about 8 miles to Loma Linda.

LABORATORY PROCEDURES FOR BLOOD CULTURE AND SLIDE PREPARATION (carried out by the Genetics Research and Chromosome Services, Loma Linda University Medical Center)

Processing of the blood samples was initiated the same day within 12 hours of the venipunctures. Using the same Macro Technique utilized by Dr. Charles Scott in his preliminary smog study in Los Angeles, the tubes of blood were centrifuged at 2000 rpm for 10 minutes to separate the blood layers. By use of sterile pipettes, most of the white blood cells were separated off (and a little plasma) from the buffy coat layer interface between the red blood cells and plasma. These were transferred into 4 tubes each containing 10 ml of media (F-10 Hank's, 20% fetal calf serum, L-glutamine, phytohemagglutinin P and antibiotics). The tubes were then refrigerated overnight at approximately 10°C. The next morning they were transferred into a body temperature 37°C incubator. Two of the tubes were incubated for 48 hours and two of the tubes were incubated for 72 hours before harvesting. The 72-hour tubes were used as back-up cultures in case the lymphocytes were slow growing and would not have obtained sufficient metaphases within the normal 48-hour cultivation period. Two hours before harvesting, the cells were mixed and 0.15 ml of 2 mcg/ml colcemid added to each tube.

At harvest the culture was centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded and the cells were mixed and 10 ml of 0.075 M KCL, warmed to 37°C, were added to each tube. The tubes were then placed in a water bath at 37°C for 10 minutes. The tubes were recentrifuged at 1500 rpm for 5 minutes, the supernatant discarded, and the cells mixed. One milliliter (1 ml) of Carnoy's fixative (3 parts methanol, 1 part acetic acid) was added slowly, then an additional 2 ml of fixative was added and solution mixed well to dissolve all clumps. Tubes were allowed to stand for 10 minutes. The contents of both tubes were combined into one, additional fixative added to fill tube, and the mixture centrifuged at 1500 rpm for 5 minutes. This procedure was repeated two more times. Then 2 drops of the final cell mixture were dropped on a clean glass slide (which had been just dipped in room temperature distilled water) from a height of approximately 2 feet.

The slides were allowed to air dry for 24 hours and then stained for 5 minutes with routine Giemsa (Harleco azure blend type Giemsa: 3 ml McIlvaines pH 6.8 and 47 ml distilled water and 1 ml of the Giemsa preparation). Slides were allowed to dry overnight before coverslipping.

METHOD OF CHROMOSOME ANALYSIS (carried out by cytogeneticists at Utah Biomedical Test Laboratory, University of Utah)

Slides were randomized, coded, and scored blind at 1000X magnification. All 100 cells were analyzed for chromosome and chromatid aberrations; however, only 25 cells of this 100 were counted for aneuploidy. The following table lists the number of students included in the study and the number for which metaphases were cytogenetically scored for each blood sampling period.

	BLOOD DRAWING			
	9/76	11/76	4/77	9/77
Number of Students Included in study	398	396	360	319
Number of Students Cytogenetically Scored	398	396	267	67
Number of Metaphases Scored	39,450	38,881	18,933	6,477

The method of chromosome analysis was as follows: a metaphase cell was determined suitable by scanning with the low power objective prior to using the oil immersion objective. For purposes of documentation and verification, the number of aneuploid metaphases and the occurrence of structural chromosome and chromatid aberrations, including breaks and fragments, were carefully recorded (see Appendix F).

For purposes of data analysis, the term "abnormal cells" includes all cells containing breaks, isochromatid breaks, fragments, isofragments, translocations, locations, dicentrics, tricentrics, pericentric inversions, rings, isodeletions, polyradials and endoreduplications. Because chromatid breaks were observed to be the most common abnormality in this study, they were not only included in the classification of abnormal cells but were analyzed separately as well. Therefore, this separate category of chromatid breaks includes isochromatid breaks, fragments and isofragments. (For description of aberrations see Glossary, page 149)

Gaps were scored separately and cells with gaps were not included in the number of abnormal cells. A gap was defined as a complete interruption of the continuity of one or both chromatids not clearly exceeding the width of a chromatid. However, if the discontinuity was larger than the width of a chromatid, the aberration was scored as a break. Isogaps and isobreaks were scored as single aberrations. Whenever the following conditions existed, an aberration was scored as a single type chromatid break and not as a chromosome type aberration: 1) an acentric fragment in a metaphase spread, 2) a diploid chromosome count, and 3) whenever it appeared that the fragment was derived from an isobreak in the same spread.

Stable changes, endoreduplication, terminal blebs, and aneuploid cells (hyperdiploid and hypodiploid) were also recorded.

The chromosome analysis scan sheet, data code key and coding form used for keypunching are shown in Appendix F.

DATA PROCESSING

Both questionnaire and laboratory data were coded, key punched, machine verified, and then a 10 percent sample hand-verified to assure accuracy. A computerized data base was then formulated for each student consisting of the questionnaire and laboratory data for each blood drawing.

TABLE 1

MAJOR SUB-GROUPS OF STUDENTS

		COLLEGE EXPOSURE	
		Low*	High
PAST EXPOSURE	Low	Group 1. Students coming from low smog areas and enrolling at PUC	Group 2. Students coming from low smog areas and enrolling at La Sierra
	High	Group 3. Students coming from South Coast Air Basin and enrolling at PUC	Group 4. Students coming from South Coast Air Basin and enrolling at La Sierra

* Criterion for low versus high exposure is explained on pages 8 and 9.

FIGURE 1. HYPOTHETICAL PROFILES OF CHROMOSOME ABERRATION RATES, IF SMOG HAS AN ACUTE EFFECT, FOR VARIOUS GROUPS OF STUDENTS ON WHICH STUDY DESIGN WAS BASED

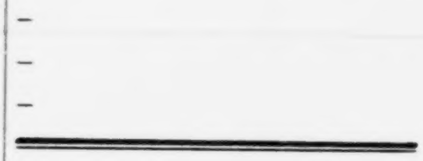
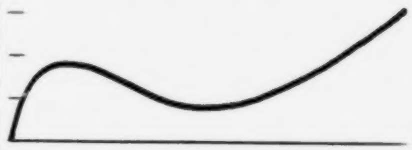
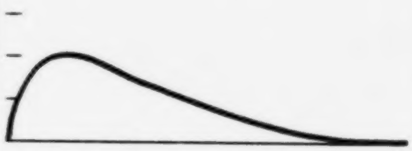
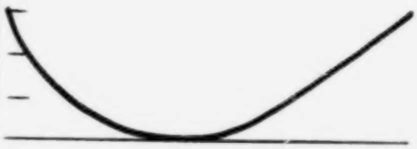
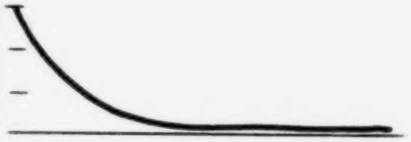
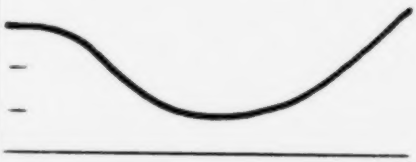
Exposure				Sep Nov Apr Sep
Past	College	Summer	Group Description	0 1 2 3 4 5 6 7 8 9 10 11 12
Low	Low	Low	Group 1: Students who stay at PUC for summer or return home for summer	
Low	High	High	Group 2: Students who stay at La Sierra for summer	
Low	High	Low	Group 2: Students who return from La Sierra to home in low smog area for summer	
High	Low	High	Group 3: Students who return from PUC to home in high smog area for summer	
High	Low	Low	Group 3: Students who stay at PUC for summer	
High	High	High	Group 4: Students who stay at La Sierra or return home to high smog area for summer	

TABLE 2

CALIFORNIA CITIES CLASSIFIED AS LOW SMOG WHICH EXCEEDED THE 300 MICROGRAMS PER CUBIC METER MAXIMUM VALUE CRITERIA					
CITY	1971 DOSAGE CLASSIFICATION	1976 MAXIMUM VALUE CLASSIFICATION	FINAL CLASSIFICATION	EXCESS DOSAGE ABOVE 0.08 ppm	MAXIMUM OBSERVATION $\mu g/m^3$
Escondido	Intermediate	High	Low	599	490
Imperial Beach	Intermediate	High	Low	114	314
Oceanside	Intermediate	High	Low	646	568
San Diego	Low	High	Low	188	333
Fresno	Low	High	Low	233	353
San Jose	Low	High	Low	338	372
Sunnyvale	Low	High	Low	108	314
Vallejo	Low	High	Low	63	372
Bakersfield	Low	High	Low	453	372
Fresno	Low	High	Low	232	431

TABLE 3

HOURS OF OZONE EXPOSURE IN EXCESS OF 0.1 AND 0.2 P.P.M AT PACIFIC UNION COLLEGE AND LOMA LINDA UNIVERSITY'S LA SIERRA CAMPUS BY MONTH

Date	P.U.C.		La Sierra	
	>0.1	>0.2	>0.1	>0.2
September 1976	2	0	116	14
October 1976	10	0	101	16
November 1976	2	0	45	3
December 1976	0	0	0	0
January 1977	0	0	2	0
February 1977	0	0	37	6
March 1977	0	0	6	0
April 1977	0	0	81	1
May 1977	0	0	39	5
June 1977	0	0	159	18
July 1977	10	0	241	36
August 1977	0	0	200	28
September 1977	0	0	98	6
October 1977	0	0	163	24
November 1977	0	0	4	0
December 1977	0	0	0	0

TABLE 4

OZONE EXPOSURES FOR PACIFIC UNION COLLEGE AND
LOMA LINDA UNIVERSITY'S LA SIERRA CAMPUS BETWEEN BLOOD DRAWINGS

P.U.C.

INCLUSIVE DATES	NUMBER OF DAYS	BLOOD DRAWING	HOURS IN EXCESS OF 0.1 ppm		HOURS IN EXCESS OF 0.2 ppm	
			Total	Average Per Day	Total	Average Per Day
Sep 2, 1976 to Sep 19, 1976	18	Before 1	2	0.11	0	0
Sep 20, 1976 to Nov 7, 1976	49	Between 1 & 2	12	0.24	0	0
Nov 8, 1976 to Apr 11, 1977	155	Between 2 & 3	0	0.00	0	0
Apr 12, 1977 to Oct 12, 1977	184	Between 3 & 4	10	0.05	0	0

LA SIERRA

INCLUSIVE DATES	NUMBER OF DAYS	BLOOD DRAWING	HOURS IN EXCESS OF 0.1 ppm		HOURS IN EXCESS OF 0.2 ppm	
			Total	Average Per Day	Total	Average Per Day
Sep 1, 1976 to Sep 26, 1976	26	Before 1	105	4.04	14	0.54
Sep 27, 1976 to Nov 14, 1976	49	Between 1 & 2	129	2.63	16	0.33
Nov 15, 1976 to Apr 17, 1977	154	Between 2 & 3	119	0.77	9	0.06
Apr 18, 1977 to Oct 20, 1977	186	Between 3 & 4	908	4.88	117	0.63

TABLE 5

MONTHLY AVERAGES OF DAILY ARITHMETIC MEANS OF POLLUTANTS AT PACIFIC UNION COLLEGE (ANGWIN) AND LOMA LINDA UNIVERSITY'S LA SIERRA CAMPUS

Year	Month	Ozone (ppm) Angwin	Ozone (ppm) La Sierra	NO^2 (ppm) Angwin	NO^2 (ppm) La Sierra	THC (ppm) Angwin	THC (ppm) La Sierra	Methane (ppm) Angwin	Methane (ppm) La Sierra	TSP (ug/m^3) Angwin	TSP (ug/m^3) La Sierra	RSP (ug/m^3) Angwin	RSP (ug/m^3) La Sierra
1976	Aug.	**	**	**	**	**	**	**	**	35.3	193.1	20.0	116.7
1976	Sept.	0.033	0.047	BMDL*	0.028	1.586	1.830	1.576	1.681	34.8	102.7	20.9	60.8
1976	Oct.	0.036	0.042	BMDL	0.040	1.816	2.026	1.766	1.775	38.6	123.4	26.4	84.9
1976	Nov.	0.029	0.036	BMDL	0.063	1.656	2.308	1.635	1.908	37.6	123.4	20.8	85.8
1976	Dec.	0.032	0.016	BMDL	0.044	1.540	1.925	1.641	1.646	38.2	95.4	28.0	63.0
1977	Jan.	0.024	0.011	BMDL	0.039	1.765	1.922	1.729	1.665	23.1	65.8	15.1	45.6
1977	Feb.	0.032	0.023	BMDL	0.055	1.835	2.202	1.748	1.862	25.7	110.9	16.1	81.3
1977	Mar.	0.036	0.026	BMDL	0.032	1.597	2.279	1.624	2.105	**	74.3	**	52.9
1977	Apr.	0.039	0.044	**	0.039	1.601	2.270	1.574	2.084	**	103.3	**	59.7
1977	May	0.033	0.038	BMDL	0.023	1.416	1.998	1.464	1.927	21.6	62.6	13.9	44.5
1977	June	0.033	0.061	BMDL	0.032	1.247	2.045	1.261	1.896	42.5	130.3	19.0	79.8
1977	July	0.036	0.063	BMDL	0.042	1.182	2.316	1.167	1.985	38.5	121.7	17.0	81.4
1977	Aug.	0.025	0.059	BMDL	0.037	1.314	2.257	1.296	2.034	36.2	124.0	11.0	73.1
1977	Sept.	0.022	0.042	BMDL	0.041	1.551	2.382	1.496	2.126	30.3	110.5	14.0	61.8
1977	Oct.	0.027	0.049	BMDL	0.056	1.639	2.763	1.558	2.407	32.3	176.7	17.4	114.3
1977	Nov.	0.019	0.029	BMDL	0.054	1.671	2.788	1.570	2.519	15.0***	159.8***	4.3***	93.8***

* Below minimum detectable level (Minimum level = 0.01 ppm)
** Air quality measurement instrument not in operation
*** Based on four observations only

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TABLE 6

MONTHLY AVERAGES OF DAILY ARITHMETIC MEANS OF OUTSIDE TEMPERATURE (OT) AND DEW POINT (DP) AT PACIFIC UNION COLLEGE (ANGWIN) AND LOMA LINDA UNIVERSITY'S LA SIERRA CAMPUS (°C)

		Angwin		La Sierra	
Year	Month	OT	DP	OT	DP
1976	Sept.	18.381	7.705	20.416	16.179
1976	Oct.	17.219	4.472	19.351	9.340
1976	Nov.	13.796	5.087	18.106	6.754
1976	Dec.	10.041	0.624	12.983	2.886
1977	Jan.	7.551	0.601	11.673	3.469
1977	Feb.	11.108	4.378	14.268	2.522
1977	Mar.	8.431	1.144	11.211	1.441
1977	Apr.	14.247	2.596	15.508	7.387
1977	May	11.619	4.415	14.639	8.964
1977	June	19.289	8.225	19.530	13.680
1977	July	20.496	7.279	23.293	13.347
1977	Aug.	20.725	8.556	23.269	17.012
1977	Sept.	17.227	7.510	21.442	14.231
1977	Oct.	15.662	7.132	18.779	12.069
1977	Nov.	10.454	5.628	19.399	3.055

CHAPTER 2

DENVER LAB VALIDITY SUB-STUDY

To validate the laboratory scoring of chromosome aberrations, a simple random sample of 10 percent of the students was selected independently from each blood drawing to be re-scored by an independent laboratory. All of the slides from these students were sent to the University of Colorado Genetics Laboratory to be scored under the direction of Dr. Herbert Lubs. Initially, Dr. Herbert Lubs and Dr. Randy Moon, the cytogeneticist from the Utah Biomedical Test Laboratory, which did the original scoring of the entire group, conferred on the scoring protocol to assure comparability. Slides were then scored independently by the University of Colorado Laboratory. There were from five to ten slides available for each student blood drawing case. All slides for each student were sent to each laboratory, but each laboratory used only enough slides to obtain 100 scorable metaphases--in some cases only one slide. Thus, although the same cases were examined, the two laboratories did not necessarily look at the same slides. The same slides that the Utah lab scored were not pre-selected for the Denver lab to allow a check on possible bias of scaling due to slide selection. This bias check was considered important because the Utah lab was using a preliminary scanning procedure to select the slides of best quality for scoring for the later blood drawings.

On Blood Drawing 1, 39 students were re-scored by the Denver laboratory. Of these 39 students, the Utah laboratory examined a total of 3,892 metaphases, the Denver laboratory 3,896. The Utah laboratory examined 100 metaphases per case for all students except one for which 92 metaphases were obtained. Likewise, the Denver laboratory examined 100 metaphases per case except for one where 96 metaphases were obtained.

On Blood Drawing 2, 39 students were re-scored by the Denver laboratory. A total of 3,888 metaphases were examined by the Utah laboratory for these students and 3,885 by the Denver laboratory. The Utah laboratory had two cases with less than 100 metaphases, one having 93 and the other 95. The Denver laboratory had one case with less than 100 metaphases, this one having 85 metaphases examined.

On Blood Drawing 3, 29 students were re-scored by the Denver laboratory. The Utah laboratory had difficulty obtaining 100 scorable metaphases on 16 of the 29 students. The distribution of number of metaphases obtained for these 16 were 6, 7, 20, 23, 24, 28, 33, 34, 40, 47, 47, 62, 84, 94, 94, and 98. The five cases with less than 25 metaphases were excluded from the paired analysis as their numbers are too small to provide stable estimates of aberration rates. The Denver laboratory had difficulty obtaining 100 metaphases for only one of these cases for which they scored 25 metaphases. Thus, for the paired analysis only 24 students were used with a total of 1,961 metaphases scored by the Utah laboratory on these students and 2,400 metaphases scored by the Denver laboratory. The scoring data was computerized and a statistical analysis conducted to compare the results.

STATISTICAL ANALYSIS

Since the same students were scored at both labs, paired t-tests were carried out for each blood drawing.

The transformation

$$Y = \sqrt{X + 1}$$

was used before pairing to improve normality. The results of the t-tests for each of the three blood drawings are shown in Tables 10 through 12. The means, standard deviations, minimums and maximums in these tables are for non-transformed values of aberrations per analyzed metaphases for ease of interpretation. The t-statistics and p values are based on transformed values.

Terminal blebs were excluded because of the highly subjective interpretative nature of this variable. All cases which had fewer than 25 metaphases that could be scored were excluded from analyses.

In looking at the p values in Tables 7 through 9, pages 30 through 32, the problem of multiple comparisons must be remembered. Since there is a total of 24 t-tests, an individual significance level of .002 would have to be used to assure an overall significance level of .05. The t-tests would suggest that the agreement between the two laboratories for the overall results of Blood Drawing 1 is good except for chromatid gaps where Utah appeared to be scoring somewhat higher than Denver. On Blood Drawing 2, Utah again appeared to be scoring slightly higher than Denver on abnormal cells, chromatid and isochromatid breaks, chromatid gaps and polyradials. In Blood Drawing 3, there was good agreement except for chromatid gaps and polyradials where Utah again was scoring slightly higher than Denver.

The above t-tests indicate whether differences between laboratories are statistically significant, but not whether the differences are cytogenetically significant. Where statistically significant differences occurred, the magnitude in mean difference was less than 2 percent so that although some differences appear significantly different on a statistical basis, the magnitude of the difference is not large for a cytogenetic study of this nature.

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CYTOGENETIC EVALUATION OF INTER-LAB VALIDITY SUB-STUDY

To compare the results of the two laboratories on a cytogenetic basis, a meeting was held between the cytogeneticists of both laboratories in which the results of the statistical analyses were reviewed as well as scoring sheets for individual cases and photographs of slides for possible differences in scoring techniques. The absolute differences of the aberration rate obtained between the two laboratories were ranked from highest to lowest for each variable, then a cut-off line at ±2 standard deviations was imposed on the ranked list and the scoring sheets for each laboratory compared for all cases above the line, that is, those with differences exceeding ±2 standard deviations.

After scoring sheets had been compared for the cases with large differences for each variable, as well as selected photographs, it was the opinion of all cytogeneticists at both laboratories that there were no biologically meaningful differences between the Utah and Denver laboratories for Blood Drawing 1. It was felt that the statistically significant difference in mean rate for gaps was due to the highly subjective nature of the interpretation of this variable. The Utah laboratory technicians were not familiar with scoring spreads of chromosomes with banded staining. Cytogeneticists at Denver were familiar with this technique and had little difficulty in scoring spreads containing such stained chromosomes. A few of the poorer quality slides had some banding which the Utah laboratory may have interpreted as gaps where the Denver laboratory did not, thus, resulting in a higher rate for gaps for the Utah laboratory. Where large discrepancies occurred on individual cases, they seemed to result mainly from the Utah laboratory scoring different slides from those scored by the Denver laboratory and the large inter-slide variability due to the rare occurrence of artifacts accounting for a major portion of the differences observed. For example, for one case in Blood Drawing 1, the Utah laboratory found two stable changes, one deletion and one dicentric after scanning 42 cells on slide A-1. All of the Denver laboratory's observations were made from a different slide, A-4, in which no aberrations were found. The Utah laboratory, in scanning 47 cells from this same slide, also found no aberrations.

On Blood Drawing 2, the Utah laboratory scored a slide which the Denver laboratory did not score which contained three dicentrics, four isofragments and two fragments. Since these all count as breaks, the break rate is greatly inflated for the Utah laboratory. On examination of other cases, it appeared that the Utah laboratory seemed to be consistently scoring chromatid breaks at a somewhat higher rate that the Denver laboratory, but again the difference was not felt to be biologically significant by the cytogeneticists. Fragments and isofragments are counted under abnormal cells, isofragments under isochromatid breaks and all breaks under aberrations per analyzed metaphase, so this same case inflated the rates for the Utah laboratory for these variables as well. There was one other case which was inflating Utah's rates consistently for these variables. For this case the Denver laboratory scored 83 of their metaphases from one slide. The Utah laboratory scored only 29 metaphases from this slide so that again discrepancies resulted from scoring different slides.

In the third blood drawing, 13 of the 14 chromatid breaks observed by Utah occurred in one cell. In this cell there were nine isofragments (nine breaks) and two dicentrics (which equals four breaks), thus giving a total of thirteen breaks for this one cell which was from a different slide than those the Denver laboratory examined. This same problem also affects isochromatid breaks explaining the higher maximum value attained by the Utah laboratory and the slightly higher mean.

After reviewing the statistical analyses and making individual comparisons of scan sheets it was the opinion of all the cytogeneticists of both labs that the differences observed were within the realm of expected interlab variability for cytogenetic scoring.

TABLE 7

BLOOD DRAWING 1: INTER-LABORATORY VALIDITY STUDY:
COMPARISON OF MEANS UTAH LABORATORY VERSUS DENVER LABORATORY
FOR EACH VARIABLE BASED ON 39 STUDENTS
100 METAPHASES PER STUDENT

	UTAH LABORATORY				DENVER LABORATORY					
VARIABLES	X%	S.D.	MIN.	MAX.	X%	S.D.	MIN.	MAX.	t	p
Abnormal Cells	1.18	1.25	0	5	0.90	1.10	0	4	1.31	0.20
Chromatid Breaks	1.28	1.47	0	6	0.67	0.90	0	3	2.29	0.03
Isochromatid Breaks	0.28	0.65	0	3	0.26	0.50	0	2	0.09	0.93
Stable Changes	0.10	0.38	0	2	0.02	0.16	0	1	1.12	0.27
Chromatid Gaps	1.75	1.39	0	4	0.38	0.71	0	2	6.05	0.00
Isochromatid Gaps	0.05	0.22	0	1	0.02	0.16	0	1	1.00	0.32
Polyradials	0	0	0	0	0	0	0	0	0	1.00
Aberrations	2.31	2.61	0	9	2.18	2.96	0	11	0.46	0.65

TABLE 8

BLOOD DRAWING 2: INTER-LABORATORY VALIDITY STUDY: COMPARISON OF MEANS UTAH LABORATORY VERSUS DENVER LABORATORY FOR EACH VARIABLE BASED ON 39 STUDENTS 100 METAPHASES PER STUDENT

VARIABLES	UTAH LABORATORY				DENVER LABORATORY					
	X%	S.D.	MIN.	MAX.	X%	S.D.	MIN.	MAX.	t	p
Abnormal Cells	1.28	1.47	0	6	0.49	0.72	0	3	3.14	0.003
Chromatid Breaks	1.56	2.29	0	12	0.46	0.72	0	3	3.32	0.002
Isochromatid Breaks	0.33	0.77	0	4	0	0	0	0	*	*
Stable Changes	0.08	0.48	0	3	0.03	0.16	0	1	0.54	0.60
Chromatid Gaps	1.75	1.54	0	5	0.69	0.89	0	3	3.92	0.00
Isochromatid Gaps	0.03	0.16	0	1	0.08	0.27	0	1	-1.00	0.32
Polyradials	0.03	0.16	0	1	0	0	0	0	*	*
Aberrations	2.92	3.88	0	15	1.13	2.00	0	9	2.66	0.01

* t-tests not computed because one group has zero variance.

TABLE 9

BLOOD DRAWING 3: INTER-LABORATORY VALIDITY STUDY:
COMPARISON OF MEANS UTAH LABORATORY VERSUS DENVER LABORATORY
FOR EACH VARIABLE BASED ON 24 STUDENTS
WITH A TOTAL OF 1,961 METAPHASES EXAMINED BY UTAH AND 2,400 BY DENVER

VARIABLES	UTAH LABORATORY				DENVER LABORATORY				t	p
	X̄	S.D.	MIN.	MAX.	X̄	S.D.	MIN.	MAX.		
Abnormal Cells	1.94	1.83	0	6	1.79	1.72	0	6	0.33	0.75
Chromatid Breaks	2.40	3.10	0	14	2.29	2.24	0	8	-0.07	0.94
Isochromatid Breaks	1.06	2.03	0	9	0.33	0.92	0	3	1.62	0.12
Stable Changes	0.08	0.41	0	2	0.21	0.51	0	2	-0.99	0.33
Chromatid Gaps	3.61	2.02	0	8	1.67	1.68	0	6	3.86	0.001
Isochromatid Gaps	0.08	0.28	0	1	0.17	0.38	0	1	-1.00	0.33
Polyradials	0	0	0	0	0.04	0.20	0	1	*	*
Aberrations	4.33	4.47	0	16	4.62	4.25	0	12	-0.25	0.80

* t-tests not computed because one group has zero variance.

CHAPTER 3

STATISTICAL ANALYSES FOR BLOOD DRAWING 1

DESCRIPTIVE CHARACTERISTICS OF STUDENTS

The initial blood drawing of students was conducted September 19 through 22 at the Pacific Union College campus and September 26 through 30 at the La Sierra campus. At the time of this first blood drawing, students completed a detailed questionnaire (see Appendix C) which ascertained possible exposure to covariates not associated with ozone exposure which might cause chromosome aberrations as well as the student's past residence history and possible short-term air pollution exposures due to vacations during the summer months. The characteristics of the entire group as ascertained by questionnaire are given in Tables 10 through 18, pages 39-46. Table 19, page 47, displays other chemicals, illnesses, and medications that were written in by the students. Table 20, page 49 , shows the distribution of covariate exposure scores by sex for each of the four main study groups, as defined in Chapter 1.

STUDY DESIGN GROUPS

There were four main study groups defined by the research design -- the "low-to-low" group (students coming from areas of little or no oxidant air pollution to Pacific Union College), the "low-to-high" (students coming from areas of little or no oxidant air pollution to the La Sierra campus), the "high-to-low" group (students coming from the high oxidant air pollution areas of the South Coast Air Basin to attend school of Pacific Union College), and the "high-to-high" group (students coming from areas of high oxidant air pollution in the South Coast Air Basin to attend school at La Sierra College).

Students who were away from home for more than two weeks during the summer months were excluded from these four main study groups and placed in groups 5 though 8 according to the type of intervening exposure. Table 21, page 50, shows the number of students in each group. These eight study design groups remain the same throughout all four blood drawings of the study with the primary analyses being conducted between the first four groups.

EXCLUSION CRITERIA

Students who came from areas of intermediate oxidant exposure or high SO_2 exposure (see definition of intermediate and high SO_2 under methodology) were excluded from analysis. In addition to this criterion, those individuals in the low-to-high group must have had a life-time residence history of low ozone exposure and must have arrived on campus not more than 15 days before blood drawing.

TRANSFORMATION OF DATA

The transformation used for all statistical tests was $Y = \sqrt{X + 1}$, where X is the number of aberrations per 100 cells. This transformation stabilizes the variance more effectively than the square root transformation, normally used for Poisson data, when some counts are small, as was the case in this data where there were a large number of zero counts, Snedecor and Cochran, (13). We also found that for this data this transformation improved the normality more than did the ordinary square root transformation. Throughout the rest of this report, this transformation is used in computing all tests of statistical significance, but means and standard deviations are given for the non-transformed data for ease of interpretation.

RESULTS

One-way analysis of variance tests were run between the four main study design groups for males and females separately to determine whether there were significant differences in mean break rates per 100 cells.

Table 22, page 51, shows the means for each type of chromosome abnormality scored for the male and female students.We see that there are no significant differences between groups for either males or females.

The variables--isochromatid gaps, polyradials, stable changes, and terminal blebs behave more like categorical variables than continuous variables with 1% or fewer of the students having values greater than 1 and 88% or more of the students having values of 0. Since the square root transformation would not improve the normality as well for these variables, chi-square tests were run in addition to the one-way analysis of variance tests. The chi-square tests compared the percentage of students with one or more aberrations for the four study design groups. Tests were run separately for males and females. None of the chi-square tests was statistically significant except for isochromatid gaps for males which had a p value of 0.02. The low-to-low group had the highest percentage of students with isochromatid gaps, 25%, compared to 0% in the low-to-high group, 155 in the high-to-low group, and 4% in the high-to-high group.

Because many statistical tests of a significance are being made throughout this study, one must bear in mind the fact that by chance alone a significance level of .05 could be seen for one out of every 20 tests. Thus, one should be cautious in rejecting a null hypothesis unless the level of significance is considerably beyond .05 and there appears to be a biological consistency of results. For the sake of brevity, throughout the rest of this report, all statements regarding statistical significance refers to the .05 level unless otherwise specified. Multiple comparison tests have been used to give an overall significance level of .05 for multiple comparisons within a particular one-way analysis of variance tests.

COVARIATE EXPOSURE ADJUSTMENT

To adjust for the possible effects of covariates not associated with ozone exposure, general linear hypotheses were run on the four groups using sex and study group as design variables and the covariate factor score. The F statistic for the covariate factor score was not significant for any of the variables in the general linear hypotheses indicating no association of the covariate factors with the aberration variables under study. Significant sex-group interactions were found however. Because of this the one-way analyses of variance were run sex-specific, first including students who had covariate exposure and then excluding students with covariate exposure. The results obtained when students with covariate exposure were excluded were in general similar to those obtained when they were included (see Table 22, page 51).

Another possible covariate could be the fact that some of the blood samples were transported by plane even though the aircraft was kept below 7,000 feet elevation as described on page 16. If this factor had an effect it would show up in the comparison of the high-to-low group vs. the high-to-high group as these groups would be expected to be the same since air pollution exposure effects would not have had time to "wash out" before blood drawing. The blood samples for the high-to-low group were transported by plane; those for the high-to-high group were not. The F tests in table 22, however, accepted the null hypotheses meaning that no pair-wise differences between groups are significant so that these groups do not differ significantly with respect to any of the types of chromosome abnormalities.

Sex-specific comparisons were made between students who had covariate exposure and students who did not have covariate exposure. For females none of the variables showed significant differences. For males only two of the variables showed statistically significant differences - chromatid gaps and aberrations per analyzed metaphase. The males with covariate exposure had a lower rate of chromatid gaps (1.52% versus 2.48% for those without exposure) and a higher rate of aberrations for analyzed metaphase (4.93% versus 3.11% for those without covariate exposure). Both differences in rates had a level of statistical significance less than .02. For the majority of covariates there were not enough students with exposure on an individual covariate to warrant separate statistical analysis for each covariate. For a few covariates however individual statistical analyses were conducted. These included past psychedelic drug use (cocaine, LSD, marijuana, and other psychedelics), past cigarette use, coffee drinking, and use of birth control pill. For each covariate sex-specific t-tests using the x + 1 transformation were conducted comparing the mean rates of aberrations for each of the variables for those with moderate to heavy exposure versus those with little or no exposure. For the past use of psychedelic drugs and cigarettes the "frequently" or "occasionally" categories were combined and compared to the "tried once or twice" or "never used" categories. For caffeine-containing beverages the categories "daily", "several times per week", and "a few times per week" were combined and compared against the categories "a few times per month", "rarely", or "never". Current users of birth control pills were compared against non-users. No significant differences were found except for caffeine-containing beverages for males on the variable isochromatid breaks where the rate was 0.67% for the moderate to heavy users versus 0.36% for the light users. The level of significance was .04

CAMPUS ARRIVAL TIME

Previous studies by Zelac (3) and Merz (7) have indicated that ozone may affect lymphocytes in a time period shorter than 15 days. Thus, to be sure that we were not masking the differences between groups by allowing too long a time period for arrival on campus before blood drawing, two additional more restrictive sets of criteria, were applied to the low-to-high smog group. The first criterion was to exclude all individuals in this group who had arrived more than three days prior to blood drawing, the second to exclude all those who had arrived more than one day prior to blood drawing. When the 3-day and 1-day criteria were applied, significant differences between groups were noted for males with the low-to-high group, having higher aberration rates for stable changes than the other study design groups (see Table 23, page 52). Increased differences were noted for females though they were not significant. The groups still did not differ significantly on the other types of chromosome abnormalities. The Scheffe Multiple F test was used to determine if the mean for males for the low-to-high group was significantly higher than the low-to-low group and/or significantly higher than the average mean of the other three groups. Of the 3-day criterion, the low-to-high group was significantly (.05 level or less) higher than the average of the means of the other three groups but not significantly different from the low-to-low group. For the 1-day criterion, both comparisons were significant. To be sure that these higher rates for the low-to-high group were not the result of covariate exposure, all students with covariate exposure were excluded and another one-way analysis of variance was run. The results are shown in Table 23. Although the number of students in the low-to-high group is small when the 1-day and 3-day criteria are applied, it can be seen from Table 23 that the aberration rate for this group becomes increasingly elevated above the other groups as the 15-day, 3-day and 1-day criteria are applied with it being most marked for the 1-day criterion. The same is true for females although the results again do not reach statistical significance. The questionnaire and blood data for the eight male students in the low-to-high group according to the 3-day criterion and the five according to the 1-day criterion were examined by hand to see if there was some possible explanation other than ozone exposure which might have caused the elevated aberration rates. Three of the eight individuals had one stable change each; the rest had none. Of the individuals who had this one stable change, one had been employed in pest control and had used pesticides extensively throughout the summer with the exposure ending at the end of August. This student had not been coded as having a covariate exposure as the criterion for pesticides to be a covariate was that the exposure be within the month of blood drawing. There appeared to be nothing in the recorded data other than ozone that could account for stable changes in the other two students. These three students who had experienced stable changes also satisfied the criterion of arriving on campus within one day of blood drawing. The one-way analysis of variance was re-run for males excluding the students with covariate exposure and case no. 1077 who had the pesticide exposure. The F statistic for the 15-day criterion was 0.88, level of significance 0.456, and the mean for the low-to-high group 0.16. The F statistic for the 3-day criterion was 1.69, level of significance 0.175 and the mean for the low-to-high group 0.28. The F statistic for the 1-day criterion was 3.38 level of significance 0.022 and the mean for the low-to-high group 0.50 with this individual excluded.

Thus, the differences between groups for the 1-day criterion are still statistically significant though they must be regarded as very tenuous because of the small sample size of only three students in the low-to-high group.

The next step was to look at the ozone exposure experienced by the nine males in the low-to-high group who met the 3-day criterion. Table 24, page 52, shows the possible ozone exposure along with the stable change rates for each student. Since the dates of arrival on campus and blood drawing differ for the students, the possible exposure also differs. It should be kept in mind that such factors as relative time indoors/outdoors or time spent away from campus by the students during these days could alter the actual exposure experienced. Values of ozone exposure are missing for some days in Table 24 because the student was not yet on campus. The missing values are probably 0 hours in excess of 0.1 ppm as the students all came from "low" areas.

It can be noted in Table 24 that the total ozone exposure for students who experienced stable changes is never more than three hours in excess of 0.1 ppm. None of the entire group of nine students experienced levels of ozone in excess of 0.2 ppm. The greatest possible exposure over the three-day period was eleven hours in excess of 0.1 ppm.

A cross-classification of students according to total ozone exposure and whether they experienced stable changes was made for the nine males in Table 24. The results are shown in Table 25, page 53. One out of the five students or 20% of those who had some ozone exposure in excess of 0.1 ppm had stable changes; whereas, two out of the four students who had no ozone exposure in excess of 0.1 ppm or 50% experienced stable changes. If the student with summer pesticide exposure and the student with other covariate exposure are excluded, the cross-classification Table 25 would have two students in the no exposure group with one having stable changes and one not having stable changes but still having a rate of 50%. Thus the rate of stable changes is higher in those students who had no ozone exposure in excess of 0.1 ppm than in the group who had exposure. Because of the very small sample sizes, it is very difficult to draw any conclusions, but there is definitely lack of conclusive evidence that ozone exposure was responsible for producing the stable changes, although there is some possibility that there may be a shock effect for some students. Because of the problems of sample size, i.e. a sample of only 100 cells from each student, and the small number of students, it would be expected to see changes only in some students even though all students were exposed to ozone. Thus the results do not rule out the possibility of a shock effect but neither do they give conclusive support to such an hypothesis.

Table 26, page 55, shows the average hours of possible ozone exposure in excess of 0.1 ppm experienced by males and females in the low-to-high group criteria: 15-day, 3-day, and 1-day. The exposures for the high-to-high group are not calculated as it is not known what exposure levels they experienced in their home areas in the South Coast Air Basin prior to arrival on campus. Since their blood was drawn at the same time as the low-to-high group, their exposures after arrival on campus should be similar to the low-to-high group.

TABLE 10

BLOOD DRAWING 1: CHARACTERISTICS OF ENTIRE GROUP OF 398 STUDENTS AS ASCERTAINED BY QUESTIONNAIRE

Covariate	Question*	Percentage of Answers		
		No	Yes	Unknown
Drug allergies	4	86.4	13.6	0
Other allergies	5	76.9	23.1	0
Food supplements	6	72.6	27.1	0.3
Aerosol products	7	56.3	43.7	0
Hobbies using glue, solvent, paint or chemicals	8	81.9	17.8	0.3
Viral infection†	16	79.9	26.1	0
Infectious mononucleosis††	17	98.0	2.0	0
Hepatitis††	18	100.0	0	0
Hay fever††	19	88.4	11.6	0
Asthma††	20	97.0	3.0	0
Illness causing rash††	21	93.5	6.5	0
Illness preventing normal activities for more than a week††	22	95.0	5.0	0
Prescription medications††	23	69.9	29.6	0.5
Non-prescription medications††	24	58.0	41.7	0.3
Vaccinations, boosters or protective serum††	25	77.4	21.6	1.0
X-radiation other than diagnostic purposes	26	97.9	1.8	0.3
Diagnostic X-rays other than routine chest and dental X-rays	27	53.3	44.7	1.0
Radioactive substances for diagnosis	28	96.5	3.5	0
Medications taken routinely for a period of more than one month	29	70.9	26.6	2.5

* For complete wording of questions, see questionnaire, Appendix C, p. 113

† Had during the last month.

††Had during the last six months.

(continued)

TABLE 10 (continued)

No.	Percent	Question
9.		How often do you shave with an electric razor or use a hand hair dryer?
	37.8	Daily
	14.1	4 or more times per week
	20.4	3 or fewer times per week
	27.4	Rarely or never
	0.3	Unknown
10.		On the average, how many hours per week do you watch television?
	3.0	16 or more hours per week
	6.3	11-15 hours per week
	21.6	6-10 hours per week
	38.4	1-5 hours per week
	30.7	Less than 1 hour per week
	0	Unknown
11.		On the average, how much sunbathing or equivalent exposure to the sun or a sun lamp do you have? (Include work or play in the sun if a considerable part of your body is exposed such as bare back, shorts, bathing suit).
	12.1	11 or more hours per week
	22.4	6-10 hours per week
	45.1	1-5 hours per week
	20.4	Less than 1 hour per week
	0	Unknown
12.		How frequently do you use cola drinks, including Dr. Pepper, Mountain Dew, etc. (Check nearest category).
	8.5	Almost every day
	16.8	A few times per week
	22.6	A few times per month
	33.7	Rarely
	18.1	Never
	0.3	Unknown

(continued)

TABLE 10 (continued)

No.	Percent	Question
13.		How frequently do you use other caffeine-containing beverages such as coffee or tea (except herb type)?
	2.3	Daily
	5.0	Several times per week
	6.5	A few times per week
	11.6	A few times per month
	25.9	Rarely
	48.7	Never
	0	Unknown
14.		How often do you eat meat?
	7.8	Daily
	10.3	Several times per week
	12.1	A few times per week
	18.8	A few times per month
	19.8	Rarely
	31.2	Never
	0	Unknown
15.		What alcoholic beverages have you ever used? Please indicate how often used.

	Frequent	Occasionally	Tried once or twice	Never Used	Unknown
Beer	0.3	9.3	21.6	68.3	0.5
Wine	0.5	7.5	27.4	64.1	0.5
Hard Liquor	0.8	6.3	14.8	76.6	1.5

No.	Percent	Question
30.		How much have you used cigarettes, cigars or pipes?
	1.5	Frequently
	4.8	Occasionally
	18.3	Tried once or twice
	75.4	Never used
	0	Unknown

(continued)

TABLE 10 (continued)

No.	Question				
31.	How frequently have you experimented with:				
	Frequently	Occasionally	Tried once or twice	Never used	Unknown
Cocaine	0.0	0.5	2.0	97.0	0.5
Marijuana	1.0	8.0	8.3	82.4	0.3
L.S.D.	0.3	1.5	1.8	95.9	0.5
Other Psychedelics	0.3	1.0	1.5	96.7	0.5
Amphetamines (stimulants)	0.3	1.0	4.0	94.2	0.5
Barbiturates (sleepers)	0.0	1.0	2.5	96.2	0.3

TABLE 11

BLOOD DRAWING 1: AMOUNT OF EXPOSURE TO OCCUPATIONAL CHEMICAL EXPOSURE--CHEMICAL 1
(Question #3)

Length of Exposure	Frequency	Percent of Total
None	262	65.9
Less than 3 months (part-time)	20	5.0
Less than 3 months (full-time)	17	4.3
3 months to 1 year (part-time)	22	5.5
3 months to 1 year (full-time)	22	5.5
1 year to 3 years (part-time)	22	5.5
1 year to 3 years (full-time)	17	4.3
More than 3 years (part-time)	10	2.5
More than 3 years (full-time)	6	1.5
	398	100.0

TABLE 12

BLOOD DRAWING 1: TYPES OF ENVIRONMENTAL ALLERGIES
(Question #5)

Allergy	Frequency	Percent of Total
None	306	76.8
Animal	16	4.0
Plant	15	3.8
Inert	6	1.5
Plant and animal	10	2.5
Animal and inert	1	0.3
Plant and inert	23	5.8
Plant, animal, and inert	21	5.3
Unknown	0	0
	398	100.0

TABLE 13

BLOOD DRAWING 1: TYPES OF FOOD SUPPLEMENTS USED (Question #6)		
Food Supplement	Frequency	Percent of Total
None	294	73.9
Vitamin A	2	0.5
Vitamin B	8	2.0
Vitamin C	16	4.0
Vitamin E	2	0.5
Multi-vitamins	69	17.3
Other	5	1.3
Unknown	2	0.5
	398	100.0

TABLE 14

BLOOD DRAWING 1: YEARS OF EXPOSURE TO MEDICATIONS (QUESTION #29)		
Length of Exposure	Frequency	Percent of Total
None	384	96.4
1 year	4	1.0
2 years	4	1.0
3 years	4	1.0
4 years	1	0.3
5 years	1	0.3
	398	100.0

TABLE 15

BLOOD DRAWING 1: TYPES OF OCCUPATIONAL CHEMICAL EXPOSURE (QUESTION #3)

Occupational Chemical Exposure	Chemical 1 Frequency	Chemical 1 % of Total	Chemical 2 Frequency	Chemical 2 % of Total
None	260	65.4	384	96.3
Cleaning solvents	18	4.5	5	1.3
Soaps and detergents	12	3.0	1	0.3
Liquid or gaseous fuels	13	3.3	1	0.3
Lacquer/paint/stain	28	7.0	0	0
Plastic solvents	6	1.5	2	0.5
Insecticides/herbicides	10	2.5	2	0.5
Chemistry lab	4	1.0	1	0.3
Other*	35	8.8	2	0.5
Unknown	12	3.0	1	0.3
Total	398	100.0	398	100.0

*See Table 19

TABLE 16

BLOOD DRAWING 1: TYPES OF DRUG ALLERGIES (QUESTION #4)

Drug Allergy	Allergy 1 Frequency	Allergy 1 % of Total	Allergy 2 Frequency	Allergy 2 % of Total
None	345	86.6	395	99.2
Antibiotics	33	8.2	2	0.5
Egg White	1	0.3	0	0
Horse serum	1	0.3	0	0
Other	17	4.3	1	0.3
Unknown	1	0.3	0	0
Total	398	100.0	398	100.0

TABLE 17

BLOOD DRAWING 1: TYPE OF PRESCRIPTION MEDICATIONS USED (QUESTION #23)

Prescription Medication	Medication 1 Frequency	Medication 1 % of Total	Medication 2 Frequency	Medication 2 % of Total
None	278	69.7	369	92.6
Birth Control Pills	11	2.8	6	1.5
Tranquilizers	13	3.3	1	0.3
Stimulants	0	0.0	1	0.3
Antibiotics	41	10.3	12	3.0
Analgesics	25	6.3	1	0.3
Decongestants	15	3.8	2	0.5
Other*	15	3.8	6	1.5
Unknown	0	0	0	0
Total	398	100.0	398	100.0

*See Table 19

TABLE 18

BLOOD DRAWING 1: TYPES OF NON-PRESCRIPTION MEDICATIONS USED (QUESTION #24)				
Non-prescription Medication	Medication 1		Medication 2	
	Frequency	% of Total	Frequency	% of Total
None	235	59.0	376	94.4
Tranquilizers	3	0.8	0	0
Stimulants	2	0.5	3	0.8
Antibiotics	0	0	2	0.5
Analgesics	139	34.9	10	2.5
Decongestants	10	2.5	3	0.8
Other*	9	2.3	4	1.0
Unknown	0	0	0	0
Total	398	100.0	398	100.0

*See Table 19

TABLE 19

WRITTEN RESPONSES FOR SELECTED QUESTIONS OF BLOOD DRAWING 1

OTHER CHEMICAL FROM TABLES

Ether
Alcohol
ZNO
HCL
Hydrogen peroxide
Graphic art supplies
Acetone
Ink
Lime
Cement
Gympsum
Gleve
Coloring
Elastron
Floor mastic
Chesadent
Jeltrate
Glue
Zinc
Lead in welding
Chromic acid
Mercury
Amalgam
Novacaine
Embalming fluid
Hydrochloric acid
Photo developing chemicals
Calcium sulfate
Asbestos
Epoxy glue
Metal dust
Kerosene
Gas
Oil

SPECIFIED ILLNESS (causing rash) FROM QUESTION 21

Weeds - September
Shingles - 1968
Measles - 1960 and 1962
Hives - December 1975
Chicken pox - 1962 and 1964
Virus - July 1976
Allergy - April 1976
Strep throat - September 1976
Poison oak - (4 cases) March 1973
(1 case) June 1973
Eczema - 1958
German measles - 1965
Insect stings - August 1976
Allergy - September 1976
(soaps, perfumes, makeup)

SPECIFIED ILLNESS (preventing normal activities) FROM QUESTION 22

Bronchitis - December 1975
Manic depression -
Flu - 1973 and Mar. 76
Chicken pox - 1962
Knee pains - June 1976
Strep throat - May 1975
Mononucleosis - August 1976
Allergies - August 1976
Reiter's syndrome - February 1976
Staph infection - May 1975
Kidney stones - July 1976
Severe headaches -
Stomach ulcer - June 1976
Measles - 1962
Mumps -
Wisdom teeth pulled -
Appendicitis - May 1976
Back strain - July 1976

(continued)

TABLE 19 (continued)

OTHER PRESCRIPTION MEDICATIONS FROM TABLE

Allergy shots
Lithium
Mycolog
Thyroid
Norflex
Somecaine
Lanoxin
Insulin

OTHER NON-PRESCRIPTION MEDICATIONS FROM TABLES

Nyquil
Cough drops
Ex-Lax
Feen-a-mint
Dristan

TABLE 20

BLOOD DRAWING 1: DISTRIBUTION OF COVARIATE SCORES BY SEX FOR EACH OF THE FOUR MAIN STUDY GROUPS

	GROUP							
	Low-Low		Low-High		High-Low		High-High	
Students	Male	Female	Male	Female	Male	Female	Male	Female
Total Number	62	62	39	49	21	32	31	49
% with No Covariates	90.4	71.0	82.0	81.7	90.5	90.6	80.6	69.4
% with Score of:								
1	4.8	1.6	7.7	0	0	0	9.7	0
2	4.8	25.8	7.7	16.3	9.5	6.3	6.5	28.6
3	0	0	2.6	0	0	0	3.2	0
4	0	1.6	0	2.0	0	3.1	0	0
5	0	0	0	0	0	0	0	2.0

TABLE 21

BLOOD DRAWING 1: NUMBER OF STUDENTS BY SEX IN EACH STUDY DESIGN GROUP			
Study Group	N Male	N Female	Total
1. Low-Low	62	62	124
2. Low-High	39	49	88
3. High-Low	21	32	53
4. High-High	31	49	80
5. Low-High-Low	3	2	5
6. Low-High-High	3	3	6
7. High-Low-Low	10	11	21
8. High-Low-High	10	11	21
Total	179	219	398

TABLE 22

BLOOD DRAWING 1: COMPARISON OF MEANS OF CHROMOSOME ABNORMALITIES (PER 100 CELLS) BY STUDY GROUP, SEX, AND INFLUENCE OF COVARIATES

Abnormality and Sex of Students	Low-Low A	Low-Low B	Low-High A	Low-High B	High-Low A	High-Low B	High-High A	High-High B
Number of Students								
Males	60	55	16	13	20	18	26	20
Females	61	44	33	27	27	24	38	27
Aberrations								
Males	3.57	3.34	3.69	3.23	3.35	3.50	3.23	3.25
Females	2.82	2.71*	3.97	4.48*	3.37	2.92*	2.42	2.37*
Abnormal Cells								
Males	1.63	1.56	1.77	1.64	1.40	1.50	1.31	1.35
Females	1.35	1.30*	1.81	2.06*	1.48	1.29*	1.12	1.23*
Chromatid Breaks								
Males	1.70	1.62	2.08	2.03	1.45	1.56	1.50	1.60
Females	1.48	1.42*	1.87	2.14*	1.63	1.38*	1.26	1.19*
Chromatid Gaps								
Males	2.17	2.16	2.27	2.33	2.15	2.33	2.77	3.39
Females	2.26	2.09	2.60	2.62	2.22	1.83	1.95	1.84
Isochromatid Breaks								
Males	0.40	0.33	0.88	1.00	0.40	0.44	0.35	0.45
Females	0.34	0.36	0.37	0.45	0.63	0.62	0.24	0.26
Isochromatid Gaps								
Males	0.27*	0.27	0*	0	0.15*	0.17	0.12*	0.15
Females	0.12	0.14	0.09	0.11	0.04	0.04	0.11	0.15
Polyradials								
Males	0	0	0	0	0	0	0	0
Females	0.03	0.02	0.03	0.04	0.04	0	0	0
Stable Changes								
Males	0.07	0.07	0.19	0.23	0	0	0.08	0.10
Females	0.05	0.05	0.03	0.04	0	0	0.03	0
Terminal Blebs								
Males	0.05	0.06	0.13	0.08	0	0	0.12	0.16
Females	0.07	0.07	0.12	0.15	0.19	0.08	0.08	0.07

A columns = all subjects tested
B columns = subjects without covariate exposure
* = .05 < P ≤ .10 as computed by F statistic for the group

TABLE 23

BLOOD DRAWING 1: COMPARISON OF GROUP MEANS OF STABLE CHANGES (PER 100 CELLS) BY STUDY GROUPS, COVARIATE EXPOSURE, AND 15, 3, AND 1-DAY CRITERIA APPLIED TO THE LOW-TO-HIGH SMOG GROUP

15-Day Criteria

	Males				Females			
	Group A		Group B		Group A		Group B	
Study Group	N	%	N	%	N	%	N	%
Low-Low	60	0.07	55	0.07	61	0.05	44	0.05
Low-High	16	0.19	13	0.23	33	0.03	27	0.04
High-Low	20	0.00	18	0.00	27	0.00	24	0.00
High-High	26	0.08	20	0.10	38	0.03	27	0.00

3-Day Criteria

	Males				Females			
	Group A**		Group B**		Group A		Group B	
Study Group	N	%	N	%	N	%	N	%
Low-Low	60	0.07	55	0.07	61	0.05	44	0.05
Low-High	9	0.33	8	0.38	23	0.04	19	0.05
High-Low	20	0.00	18	0.00	27	0.00	24	0.00
High-High	26	0.08	20	0.10	38	0.03	27	0.00

1-Day Criteria

	Males				Females			
	Group A**		Group B**		Group A		Group B	
Study Group	N	%	N	%	N	%	N	%
Low-Low	60	0.07	55	0.07	61	0.05	44	0.05
Low-High	5	0.60	5	0.60	9	0.11	8	0.13
High-Low	20	0.00	18	0.00	27	0.00	24	0.00
High-High	26	0.08	20	0.10	38	0.03	27	0.00

Group A = All subjects tested
Group B = Subjects without covariate exposure
** $P \leq .05$ as computed by F statistic for the group

TABLE 24

INDIVIDUAL STABLE CHANGE RATES AND OZONE EXPOSURE BY DAY FOR THE NINE MALES IN THE LOW-HIGH SMOG GROUP WHO HAD ARRIVED ON CAMPUS WITHIN 3 DAYS OF BLOOD DRAWING 1

CASE NO.	MEAN STABLE CHANGES PER 100 CELLS	DATE ARRIVED ON CAMPUS	DATE OF BLOOD DRAWING	HOURS OZONE IN EXCESS OF 0.1 ppm ON DAY PRIOR TO BLOOD DRAWING: 0	1	2	3	TOTAL
1024	1.00	Sep 26	Sep 27	3	0	–*	–	3
1028	1.00	Sep 26	Sep 26	0	–	–	–	0
1077†	1.00	Sep 26	Sep 26	0	–	–	–	0
1074	0	Sep 26	Sep 26	0	–	–	–	0
1153	0	Sep 26	Sep 27	3	0	–	–	3
1182	0	Sep 26	Sep 29	0	8	3	0	11
1250	0	Sep 26	Sep 28	8	3	0	–	11
1518	0	Sep 26	Sep 29	0	8	3	0	11
1057‡	0	Sep 24	Sep 26	0	0	0	–	0

* Student not yet on campus
† Student with summer pesticide exposure
‡ Student with other covariates

TABLE 25

CROSS CLASSIFICATION OF TOTAL OZONE EXPOSURE AND STABLE CHANGES FOR THE NINE MALES IN THE LOW-HIGH GROUP WHO HAD ARRIVED ON CAMPUS WITHIN 3 DAYS OF BLOOD DRAWING 1

		EXPOSURE		
		Yes	No	Total
STABLE CHANGES	Yes	1	2	3
	No	4	2	6
	Total	5	4	9

TABLE 26

MEAN OZONE EXPOSURE IN EXCESS OF 0.1 ppm BY DAY PRIOR TO BLOOD DRAWING 1 AT LA SIERRA CAMPUS

GROUP	ARRIVED WITHIN	NO. IN GROUP	AVERAGE HOURS PER PERSON PER DAY ON DAY:					AVERAGE HOURS PER DAY PER PERSON	TOTAL HOURS PER PERSON
			0	1	2	3	4-15		
Males: Low-High	15 days	16	1.75	2.88	0.94	0	0.54	0.75	12.00
	3 days	9	1.56	2.11	0.67	0	--*	1.08	4.33
	1 day	5	1.20	0	--	--	--	0.60	1.20
Females: Low-High	15 days	39	1.54	2.10	1.23	0.97	0.45	0.70	11.23
	3 days	23	2.48	2.87	1.13	0	--	1.59	6.35
	1 day	9	0.67	0	--	--	--	0.33	0.67

*Exposure not available as students not yet on campus but probably less than 0.1 ppm.

CHAPTER 4

STATISTICAL ANALYSES FOR BLOOD DRAWING TWO

DESCRIPTIVE CHARACTERISTICS OF STUDENTS

The second blood drawing of students was conducted November 7 through November 10, 1976 at the Pacific Union College Campus and November 14 through November 19, 1976 at the La Sierra Campus with the exception of one student in group 2 whose blood was drawn on November 30, 1976. At the time of the second blood drawing, students completed an update questionnaire (see Appendix D) which ascertained possible covariates not associated with ozone exposure which might cause chromosome aberrations as well as possible short-term air pollution exposures due to their being away from campus. The characteristics of the entire group, as ascertained by this update questionnaire are given in Table 27, page 59. Tables 28 through 32, pages 61 through 62, indicate chemicals, illnesses and medications to which the students were exposed. Table 33, page 63, shows the numbers of students by sex which were still in each of the study design groups by the November blood drawing. Table 34, page 64, shows the distribution of covariate exposure scores by sex for each of the four main study groups.

EXCLUSION CRITERIA

There were seven students who were away from campus for more than three days between the September and November blood drawings. Two of the students were in the high-to-low group at PUC (low) campus. One had been in a high smog area for five days immediately before blood drawing. This student was excluded from analysis. The other had been in Glendale, a high smog area, for four days, 27 days prior to blood drawing. This student was included in the analyses because more than two weeks had elapsed since exposure. Two additional students in group 7 (high-to-low with intervening low exposure during the summer) and who attended the PUC campus were excluded from analyses. Both students had been in a high smog area for four days, 12 days prior to blood drawing. Three students in the low-to-high group attending the La Sierra campus who had been away from campus in smog-free areas for more than three days were left in the analyses, as it was felt that the duration of time in the smog-free areas was not long enough to wash out the effects of ambient exposure at the La Sierra campus. One student had been in a smog-free area for four days, 30 days prior to blood drawing; one in a smog-free area, for four days, 21 days prior to blood drawing; and one in a smog-free area for six days, three days prior to blood drawing.

RESULTS

One-way analysis of variance tests were run between the four main study design groups for males and females separately to determine whether there were significant differences in mean break rates per 100 cells. Table 35, page 65, shows the means for each type of chromosome abnormality scored for the male and female students. We see that there are no significant differences between groups for either males or females. As in Blood Drawing 1, sex specific chi-square tests were also run for isochromatid gaps, polyradials, stable changes and terminal blebs. Again, no significant differences between groups were found. The results obtained when students with covariate exposures were excluded were in general similar to those obtained when they were included. (See Table 35).

Since the intervening exposure experienced by students during the summer months should no longer make a difference by the time of the November blood drawing, those students with intervening exposure -- groups 5 through 8 were combined with groups 1 through 4 respectively, and the sex specific one-way analysis of variance tests repeated for each variable both including and excluding students with covariate exposure. None of the tests were statistically significant.

CAMPUS ARRIVAL TIME

Since it was noted in Blood Drawing 1 that the low-to-high group had an elevated rate of stable changes which was statistically significant when the successively more restrictive criteria of 3-days and then 1-day of arrival on campus before blood drawing were applied (See Table 23), the one-way analysis of variance was repeated for Blood Drawing 2 on the same groups as Blood Drawing 1 according to the 3-day and then 1-day criterion. The results are shown for both males and females in Table 36, page 66. It can be seen from Table 36 that there are no longer significant differences between groups by the time of the second blood drawing, with the low-to-high group having an even lower rate of stable changes than the other groups. This lends additional support to the "shock hypothesis" discussed in Chapter 3. The one-way analysis of variance tests of Table 36 were re-run excluding those students with covariate exposure. Again, none of the F-statistics was significant and group means were very similar.

Another analysis compared the change in the rate of stable changes -- Blood Drawing 2 minus Blood Drawing 1 -- for each of the four subgroups. The results are shown in Table 37, page 67. We see that there is a statistically significant reduction in the rate of stable changes for the low-to-high group for the one-day criterion and close to a statistically significant reduction for the 3-day criterion for males. There is no significant change for females. This analysis was repeated excluding students who had covariate exposure prior to either blood drawing. These results are also shown in Table 37. When students with covariate exposure are excluded, the males with the 3-day criterion show a significant reduction also. Due to the small sample sizes in the 3-day and 1-day groups, one must interpret these results with caution.

Analyses of Change: Blood Drawing 2 - Blood Drawing 1

Statistical analyses of change (Blood Drawing 2 minus Blood Drawing 1) were also conducted for the other variables. These analyses are paired in the sense that they take the change in rate for each student (Blood Drawing 2 minus Blood Drawing 1) and then one-way analysis of variance tests are conducted on the change. The variable used for statistical tests of change is the difference

$$d = y_2 - y_1,$$

where

$$y_2 = \sqrt{x_2 + 1}$$

and

$$y_1 = \sqrt{x_1 + 1}$$

with x_2 being the aberration rate of Blood Drawing 2 and x_1 being the aberration rate of Blood Drawing 1.

The one-way analysis of variance tests for change (Blood Drawing 2 minus Blood Drawing 1) are shown for male and female students in Table 38, page 68. None of the differences between groups was statistically significant. These analyses were repeated excluding students who had covariate exposure prior to either blood drawing. These results for males and females are also shown in Table 38. Again, none of the tests was statistically significant. For ease of interpretation, the means displayed in Table 38 are for the mean change in the non-transformed rates per 100 cells, i.e. $x_2 - x_1$. It will be noted that there is an overall drop in rates from blood drawing one to blood drawing drawing two. Since there is not a statistically significant difference in drop between the study design groups, this drop cannot be attributed to air pollution exposure. This drop underscores the importance of having a control group in a low exposure area to control for such temporal changes. The question of what factors are causing the temporal changes is open to conjecture.

TABLE 27

BLOOD DRAWING 2: CHARACTERISTICS OF ENTIRE GROUP OF 396 STUDENTS AS ASCERTAINED BY QUESTIONNAIRE

Covariate*	Question†	Percentage of Answers		
		No	Yes	Unknown
Chemicals such as cleaning solutions other than common household cleaning agents) organic solvents, pesticides, glue, paint, etc.	3	85.1	13.0	1.0
Food supplements	4	59.1	39.9	1.0
Infections or illnesses	5	60.1	39.6	0.3
Prescription medications	6	75.2	23.5	1.3
Non-prescription medications	7	75.0	22.2	2.8
Vaccinations, boosters or protective serum	8	80.8	18.2	1.0
X-rayed	9	96.4	3.3	0.3

* Since last blood sampling (September, 1976).

† For complete wording of questions, see questionnaire, Appendix D, p. 133.

SINCE YOUR LAST BLOOD SAMPLING (SEPTEMBER, 1976) HAVE YOU:

No.	Question
10.	Used cigarettes, cigars or pipes?

2.0	Frequently
1.8	Occasionally
3.3	Tried once or twice
91.1	Never used
1.8	Unknown

(continued)

TABLE 27 (continued)

SINCE YOUR LAST BLOOD SAMPLING (SEPTEMBER, 1976) HAVE YOU:

11. Experimented with:

	Frequently	Occasionally	Tried once or twice	Never used	Unknown
Cocaine	0	0	0.3	93.1	6.6
Marijuana	0.5	1.3	2.8	91.6	3.8
L.S.D.	0	0	0	93.2	6.8
Other psychadelics	0	0	0	93.2	6.8
Amphetamines (Stimulants)	0	0.3	0.3	92.8	6.6
Barbituates (Sleepers)	0	0	0.3	92.9	6.8

12. Been away from campus for more than three days?

88.4	No
8.3	Yes, No Smog
2.8	Yes, Smog
0.5	Unknown

TABLE 28

BLOOD DRAWING 2: TYPES OF CHEMICAL EXPOSURE--CHEMICAL 1 (QUESTION #3)		
Chemical Exposure	Frequency	Percent of Total
None	337	85.1
Minimal exposure*	23	5.8
Cleaning solvents	0	0
Soaps and detergents	1	0.3
Liquids or gaseous fuels	0	0
Lacquer/paint/stain	6	1.5
Plastic solvents/glues	0	0
Insecticides/herbicides	1	0.3
Chemistry lab	22	5.6
Other	3	0.7
Unknown	3	0.7
	396	100.0

*Less than 1 hour/week

TABLE 29

BLOOD DRAWING 2: TYPES OF FOOD SUPPLEMENTS USED (QUESTION #4)		
Food Supplement	Frequency	Percent of Total
None	234	59.1
Vitamin A	3	0.8
Vitamin B	3	0.8
Vitamin C	39	9.8
Vitamin E	2	0.5
Multi-Vitamins	92	23.2
Other	9	2.3
Unknown	14	3.5
	396	100.0

TABLE 30

BLOOD DRAWING 2: TYPES OF ILLNESSES HAD (QUESTION #5)				
	Illness 1		Illness 2	
Illness	Frequency	% of Total	Frequency	% of Total
None	238	60.1	390	98.5
Viral	136	34.3	6	1.5
Non-viral	9	2.3	0	0
Other	2	0.5	0	0
Unknown	11	2.8	0	0
Total	396	100.0	396	100.0

TABLE 31

BLOOD DRAWING 2: TYPES OF PRESCRIPTION MEDICATIONS USED (QUESTION #6)				
	Medication 1		Medication 2	
Prescription Medication	Frequency	% of Total	Frequency	% of Total
None	296	74.7	375	94.6
Birth Control Pills	9	2.3	4	1.0
Tranquilizers	6	1.5	0	0
Stimulants	1	0.3	0	0
Antibiotics	39	9.8	5	1.3
Analgesics	9	2.3	1	0.3
Decongestants	12	3.0	3	0.8
Other	24	6.1	8	2.0
Unknown	0	0	0	0
Total	396	100.0	396	100.0

TABLE 32

BLOOD DRAWING 2: TYPES OF NON-PRESCRIPTION MEDICATIONS USED (QUESTION #7)				
	Medication 1		Medication 2	
Non-prescription Medication	Frequency	% of Total	Frequency	% of Total
None	297	75.0	387	97.6
Tranquilizers	1	0.3	1	0.3
Stimulants	3	0.8	1	0.3
Antibiotics	0	0	0	0
Analgesics	78	19.7	4	1.0
Decongestants	2	0.5	2	0.5
Other	14	3.5	1	0.3
Unknown	1	0.2	0	0
Total	396	100.0	396	100.0

TABLE 33

BLOOD DRAWING 2: NUMBER OF STUDENTS BY SEX IN EACH OF THE STUDY DESIGN GROUPS			
Study Group	N Male	N Female	Total
1. Low-Low	62	62	124
2. Low-High	39	48	87
3. High-Low	21	31	52
4. High-High	31	49	80
5. Low-High-Low	3	2	5
6. Low-High-High	3	3	6
7. High-Low-Low	10	11	21
8. High-Low-High	10	11	21
Total	179	217	396

TABLE 34

BLOOD DRAWING 2: DISTRIBUTION OF COVARIATE SCORES BY SEX FOR EACH OF THE FOUR MAIN STUDY GROUPS

Students	GROUP							
	Low-Low		Low-High		High-Low		High-High	
	Male	Female	Male	Female	Male	Female	Male	Female
Total Number	62	62	39	48	21	31	31	49
% with no Covariates	91.8	87.1	89.7	75.0	90.9	93.5	100	83.8
% with Score of:								
1	0	0	5.1	0	4.5	0	0	0
2	8.2	12.9	5.1	20.8	4.5	6.5	0	12.2
3	0	0	0	2.1	0	0	0	2.0
4	0	0	0	2.1	0	0	0	2.0

TABLE 35

BLOOD DRAWING 2: COMPARISON OF MEANS OF CHROMOSOME ABNORMALITIES (PER 100 CELLS) BY STUDY GROUP, SEX, AND INFLUENCE OF COVARIATES*

Abnormality and Sex of Students	Low-Low		Low-High		High-Low		High-High	
	A	B	A	B	A	B	A	B
Number of Students								
Males	59	55	15	12	21	19	26	26
Females	60	52	32	23	26	24	37	30
Aberrations								
Males	3.39	3.13	1.07	1.25	2.38	2.47	2.65	2.65
Females	2.52	2.42	2.59	2.70	3.46	3.71	2.19	2.17
Abnormal Cells								
Males	1.20	1.16	0.73	0.83	1.00	1.00	1.13	1.13
Females	1.27	1.29	1.31	1.35	1.40	1.48	1.12	1.08
Chromatid Breaks								
Males	1.52	1.46	0.73	0.83	1.29	1.32	1.20	1.20
Females	1.37	1.36	1.41	1.48	1.60	1.70	1.14	1.11
Chromatid Gaps								
Males	1.54	1.56	1.74	1.58	1.77	1.80	1.82	1.82
Females	1.45	1.41	1.75	1.61	1.94	1.94	1.99	2.04
Isochromatid Breaks								
Males	0.48	0.49	0.07	0.08	0.38	0.37	0.19	0.19
Females	0.32	0.27	0.31	0.26	0.36	0.39	0.22	0.17
Isochromatid Gaps								
Males	0.10	0.09	0	0	0.05	0	0	0
Females	0.05	0.06	0.10	0.09	0.16	0.17	0.05	0.03
Polyradials								
Males	0.02	0.02	0	0	0.05	0.05	0	0
Females	0	0	0.03	0.04	0	0	0.03	0.03
Stable Changes								
Males	0.10	0.09	0	0	0.10	0.11	0.04	0.04
Females	0.08	0.10	0.03	0.04	0.08	0.09	0	0
Terminal Blebs								
Males	0.18	0.20	0.07	0.08	0.10	0.11	0.28	0.28
Females	0.15	0.15	0.09	0.09	0.15	0.17	0.05	0.03

A columns = all subjects tested
B columns = subjects without covariate exposure
* P > .10 as computed by F statistic

TABLE 36

BLOOD DRAWING 2: COMPARISON OF GROUP MEANS PER 100 CELLS FOR STABLE CHANGES OF MALE AND FEMALE STUDENTS FOR EACH OF THE FOUR STUDY GROUPS ACCORDING TO THE 15-DAY, 3-DAY AND 1-DAY CRITERIA APPLIED TO THE LOW-TO-HIGH GROUP

15-Day	N		Males %	N		Females %
Low-Low	59		0.10	60		0.08
Low-High	15		0	32		0.03
High-Low	21		0.10	26		0.08
High-High	26		0.04	37		0
F Statistic	--	0.49	--	--	1.29	--
Level of Significance	--	0.69	--	--	0.28	--
3-Day						
Low-Low	59		0.10	60		0.08
Low-High	9		0	22		0.04
High-Low	21		0.10	26		0.08
High-High	26		0.04	37		0
F-Statistic	--	0.37	--	--	1.11	--
Level of Significance	--	0.78	--	--	0.35	--
1-Day						
Low-Low	59		0.10	60		0.08
Low-High	5		0	9		0
High-Low	21		0.10	26		0.08
High-High	26		0.04	37		0
F Statistic	--	0.28	--	--	1.32	--
Level of Significance	--	0.84	--	--	0.27	--

TABLE 37

BLOOD DRAWING 2 - BLOOD DRAWING 1: COMPARISON OF GROUP MEANS OF STABLES CHANGES (PER 100 CELLS) BY STUDY GROUPS, COVARIATE EXPOSURE, AND 15, 3, AND 1-DAY CRITERIA APPLIED TO THE LOW-TO-HIGH SMOG GROUP

15-Day Criteria

	Males				Females			
	Group A		Group B		Group A		Group B	
Study Group	N	%†	N	%†	N	%†	N	%†
Low-Low	59	0.03	50	0.02	60	0.03	38	0.003
Low-High	15	-0.20	10	-0.30	32	0	19	0
High-Low	20	0.10	17	0.12	27	0.08	24	0.09
High-High	26	-0.04	20	-0.05	37	-0.03	23	0

3-Day Criteria

	Males				Females			
	Group A *		Group B **		Group A		Group B	
Study Group	N	%†	N	%†	N	%†	N	%†
Low-Low	59	0.03	50	0.02	60	0.03	38	0.003
Low-High	9	-0.33	6	-0.50	22	0	13	0
High-Low	20	0.10	17	0.12	27	0.08	24	0.09
High-High	26	-0.04	20	-0.05	37	-0.03	23	0

1-Day Criteria

	Males				Females			
	Group A **		Group B **		Group A		Group B	
Study Group	N	%†	N	%†	N	%†	N	%†
Low-Low	59	0.03	50	0.02	60	0.03	38	0.003
Low-High	5	-0.60	3	-1.00	9	-0.11	7	0
High-Low	20	0.10	17	-0.12	27	0.08	24	0.09
High-High	26	-0.04	20	-0.05	37	-0.03	23	0

Group A = All subjects tested
Group B = Subjects without covariate exposure
* = $.05 < P \leq .10$ as computed by F statistic for the group
** = $P \leq .05$ as computed by F statistic for the group
† = Percent change Blood Drawing 2 - Blood Drawing 1

TABLE 38

BLOOD DRAWING 2 - BLOOD DRAWING 1: COMPARISON OF MEANS OF CHROMOSOME ABNORMALITIES (PER 100 CELLS) BY STUDY GROUP SEX AND INFLUENCE OF COVARIATES

Abnormality and Sex of Students	Low-Low		Low-High		High-Low		High-High	
	A	B	A	B	A	B	A	B
Number of Students								
Males	59	50	15	10	20	17	26	20
Females	60	38	32	19	27	24	37	23
Aberrations								
Males	-0.19	-0.24	-2.73	-2.70	-1.00	-1.12	-0.58	-0.50
Females	-0.17	0.08*	-1.44	-2.63*	0.07	0.92*	-0.22	0*
Abnormal Cells								
Males	-0.42	-0.36	-1.07	-1.20	-0.45	-0.59	-0.18	-0.24
Females	-0.03	0.10*	-0.52	-1.08*	-0.06	0.27*	0.02	0.01*
Chromatid Breaks								
Males	-0.17	-0.0[illegible]	-1.41	-1.70	-0.20	-0.29	-0.30	-0.38
Females	-0.07	-0.01*	-0.49	-1.06*	-0.01	0.40*	-0.09	0.10*
Chromatid Gaps								
Males	-0.66	-0.66	-0.34	-0.12	-0.29	-0.75	-0.94	-1.57
Females	-0.83	-0.58	-0.83	-1.39	-0.35	-0.07	0.09	-0.07
Isochromatid Breaks								
Males	0.08	0.16*	-0.87	-1.20*	0	-0.06*	-0.15	-0.25*
Females	-0.01	-0.13	-0.07	-0.28	-0.28	-0.24	-0.03	-0.04
Isochromatid Gaps								
Males	-0.17	-0.18	0	0	-0.10	-0.18	-0.12	-0.15
Females	-0.07	-0.08	0.00	0	0.12	0.09	-0.03	-0.09
Polyradials								
Males	0.02	0.02	0	0	0.05	0.06	0	0
Females	-0.04	-0.03	0	0	-0.04	0	0.03	0.04
Stable Changes								
Males	0.03	0.02	-0.20	-0.30	0.10	0.12	-0.04	-0.05
Females	0.03	0	0	0	0.08	0.09	-0.03	0
Terminal Blebs								
Males	0.15	0.18	-0.07	0	0.10	0.12	0.16	0.16
Females	0.07	0.08	-0.03	-0.16	-0.14	0.08	-0.03	-0.09

A columns = all subjects tested

B columns = subjects without covariate exposure

* = .05 < P ≤ .10 as computed by F statistic for the group

CHAPTER 5

STATISTICAL ANALYSES FOR BLOOD DRAWING 3

DESCRIPTIVE CHARACTERISTICS OF STUDENTS

The third blood drawing was conducted April 10, 1977, through April 13, 1977, at the Pacific Union College campus and April 17, 1977 through April 20, 1977, at the La Sierra campus. At the time of blood drawing, students again completed a questionnaire (see Appendix D) to ascertain possible covariates not associated with ozone exposure and short-term air pollution exposures due to trips away from campus. The characteristics of the entire group as ascertained by the questionnaire are shown in Table 39, page 72. Table 40, page 74, shows the number of students who had been away from campus for more than three days; and Table 41, page 74, shows the number of students who changed schools since the previous blood drawing in November. Tables 42 through 47, pages 74 to 76, show other chemicals, illnesses and medications that are written in by the students.

Due to difficulties within the Utah laboratory, complete scoring of metaphases was not attained for Blood Drawing 3. Thus, all results should be interpreted with caution for this blood drawing as it is possible that the slides scored are not a representative sample from each of the study subgroups; although, since the technicians had no way of knowing which study subgroups each of the slides were from this possibility seems unlikely. Table 48, page 77, shows the number of students with incomplete scoring by sex for each of the eight study groups. All students who did not have at least 25 metaphases scored were excluded from statistical analyses. Table 48 also shows the percentage of students for each study subgroup which had at least 25 metaphases scored. Only students who had at least 25 metaphases scored were included in the statistical analyses for Blood Drawing 3, as it was felt that fewer than 25 metaphases would provide too unreliable an indicator of aberration rates. This left only 223 out of 356 students sampled who could be statistically analysed. This same criterion was used for Blood Drawings 1 and 2.

In Blood Drawing 1 there were 398 students included in statistical analyses. Three hundred seventy-two of these students had 100 metaphases scored, 15 had 90 to 99 metaphases scored, 1 had 56 metaphases scored, and 1 had 43 metaphases scored. In Blood Drawing 2 there were 396 students. Three hundred seventy-one of these students had 100 metaphases scored, 9 had 90 to 99 metaphases scored, 8 had 80 to 89 metaphases scored, 4 students had the following respective number of metaphases scored -- 71, 70, 66, 39, and 4 students had no metaphases scored. These last four were excluded from statistical analyses.

EXCLUSION CRITERIA

Students who switched campuses between Blood Drawings 2 and 3 were excluded from analyses. In addition, students must not have been in an opposing air pollution area for more than three days within thirteen days of blood drawing for more than a total of thirty days between the November and April blood drawings. In addition to these exclusion criteria, the exclusion criteria of Blood Drawing 1 (see Chapter 3, page 34) were also applied. A 13 day criterion rather than the 15-day criterion of blood drawing one was applied here because the 15 days would have extended into the spring vacation when many students were away.

RESULTS

One-way analyses of variance were run between the four main study design groups for males and females separately to determine whether there were significant differences in mean break rates per 100 cells. Table 49, page 78, shows the means for each type of chromosome abnormality scored for the male and female students. We see that there are no significant differences between groups for either males or females. The results obtained when students with covariate exposure were excluded were in general similar to those obtained when they were included, although the significance level was slightly significant being at the 0.02 level for stable changes and chromatid gaps for males (see Table 49).

The one-way analyses of variance for change (Blood Drawing 3 minus Blood Drawing 1) are shown for male and female students in Table 50, page 79. None of the differences between groups was statistically significant except for isochromatid gaps which had a significance level slightly beyond the 0.05 level. The mean change showed a greater increase in break rates for isochromatid gaps for the males in the low-to-high group. In view of the many statistical tests run, it is likely that this represents statistical chance rather than real differences. These analyses were repeated excluding students who had covariate exposure prior to either blood drawing (see Table 50). Results were in general similar to those obtained when covariate exposures were included with isochromatid gaps, again having a significance level of 0.03 for males. The one-way analysis of variance for aberrations per analyzed metaphase for females was also slightly significant ($p = 0.04$). It will be noted that there is an overall increase in rates from blood drawing one to blood drawing 3 but since there is no statistically significant difference in increase between the study design groups, the increase cannot be attributed to air pollution exposure. As for the previous blood drawing, sex specific chi-square tests were also run for isochromatid gaps, polyradials, stable changes and terminal blebs. Again, no significant differences between groups were found.

CAMPUS ARRIVAL TIME

The variable, stable changes, was evaluated according to the 15-day, 3-day, and 1-day criteria used in Blood Drawing 1 and 2. The results are shown in Table 51, page 80. None of the groups is significantly different indicating that the initial elevation in the rate of stable changes for the low-to-high group, which dropped subsequently at the time of Blood Drawing 2, has still remained the same as the other groups at the time of Blood Drawing 3. Because of the very small numbers in the low-to-high group, however, these results can only be viewed as tentative. The analysis was not repeated for the subgroup of students without covariate exposure because of the small sample size.

TABLE 39

BLOOD DRAWING 3: CHARACTERISTICS OF ENTIRE GROUP OF 360 STUDENTS AS ASCERTAINED BY QUESTIONNAIRE

Covariate*	Question†	Percentage of Answers		
		No	Yes	Unknown
Chemicals such as cleaning solutions (other than common household cleaning agents) organic solvents solvents, pesticides, glue, paint, etc.	3	82.5	16.1	1.4
Food supplements	4	60.3	38.6	1.1
Infections or illnesses	5	49.5	49.4	1.1
Prescription mediciations	6	67.5	30.0	2.5
Non-prescription medications	7	69.5	29.4	1.1
Vaccination, boosters or protective serum	8	92.2	7.0	0.8
X-rayed	9	87.7	11.7	0.6

* Since last blood sampling (November, 1976).

† For complete wording of questions, see questionnaire, Appendix D p. 133.

SINCE YOUR LAST BLOOD SAMPLING (NOVEMBER, 1976) HAVE YOU:

No.	Question
10.	Used cigarettes, cigars or pipes?

1.1	Frequently
1.4	Occasionally
2.5	Tried once or twice
93.1	Not used
1.9	Unknown

(continued)

TABLE 39 (continued)

SINCE YOUR LAST BLOOD SAMPLING (NOVEMBER, 1976) HAVE YOU:

11. Experimented with:

	Frequently	Occasionally	Tried once or twice	Not used	Unknown
Cocaine	0	0	0	93.6	6.4
Marijuana	0.6	1.9	3.6	89.7	4.2
L.S.D.	0	0	0.3	93.0	6.7
Other psychadelics	0	0	0	93.3	6.7
Amphetamines (Stimulants)	0	0.6	0	92.5	6.9
Barbituates (Sleepers)	0	0.3	0.3	93.0	6.4

12. Been away from campus for more than three days?

13.6	No
46.4	Yes, No Smog
27.5	Yes, Smog
12.5	Unknown

TABLE 40

BLOOD DRAWING 3: BEEN AWAY FROM CAMPUS FOR MORE THAN THREE DAYS (QUESTION #12)

Absence from Campus	Frequency	Percent of Total
No	49	13.6
Yes, no smog area	167	46.4
Yes, South Coast Air Basin	99	27.5
Location not specified	43	11.9
Unknown	2	0.6
	360	100.0

TABLE 41

BLOOD DRAWING 3: CHANGED SCHOOLS SINCE NOVEMBER (QUESTION # 2)

School Change	Frequency	Percent of Total
No	355	98.5
PUC to La Sierra College	2	0.6
La Sierra College to PUC	0	0
Another College	1	0.3
Not in school now	0	0
Unknown	2	0.6
	360	100.0

TABLE 42

BLOOD DRAWING 3: TYPES OF FOOD SUPPLEMENTS USED (QUESTION #4)

Food Supplement	Frequency	Percent of Total
None	217	60.3
Vitamin A	3	0.8
Vitamin B	2	0.6
Vitamin C	37	10.3
Vitamin E	2	0.6
Multi-Vitamins	87	24.2
Other	8	2.2
Unknown	4	1.0
	360	100.0

TABLE 43

BLOOD DRAWING 3: TYPES OF CHEMICAL EXPOSURE--CHEMICAL 1
(QUESTION #3)

Chemical Exposure	Frequency	Percent of Total
None	297	82.5
Cleaning solvents	4	1.1
Soap and detergents	3	0.8
Liquid or gaseous fuels	2	0.6
Lacquer/paint/stain	16	4.4
Plastic Solvents/glues	4	1.1
Insecticides/herbicides	1	0.3
Chemistry lab	20	5.6
Other	9	2.5
Unknown	4	1.1
	360	100.0

TABLE 44

BLOOD DRAWING 3: NUMBER OF WEEKS OF EXPOSURE FOR CHEMICAL USED LONGEST
(QUESTION #3)

Length of Exposure	Frequency	Percent of Total
None	297	82.5
0 Weeks	12	3.3
1 Week	13	3.6
2 Weeks	4	1.1
3 Weeks - 7 Weeks	0	0
8 Weeks	1	0.3
9 Weeks	11	3.1
Unknown	22	6.1
	360	100.0

TABLE 45

BLOOD DRAWING 3: TYPES OF ILLNESSES HAD
(QUESTION #5)

	Illness 1		Illness 2	
Illnesses	Frequency	% of Total	Frequency	% of Total
None	178	49.5	178	49.5
Viral	149	41.4	2	0.6
Non-viral	8	2.2	3	0.8
Other	22	6.1	4	1.1
Unknown	3	0.8	173	48.0
Total	360	100.0	360	100.0

TABLE 46

BLOOD DRAWING 3: TYPES OF PRESCRIPTION MEDICATIONS USED
(QUESTION #6)

	Medication 1		Medication 2	
Prescription Medication	Frequency	% of Total	Frequency	% of Total
None	243	67.5	243	67.5
Birth Control Pills	10	2.8	0	0
Tranquilizers	8	2.2	0	0
Stimulants	0	0	0	0
Antibiotics	49	13.6	3	0.8
Analgesics	12	3.3	2	0.6
Decongestants	8	2.2	4	1.1
Other	23	6.4	8	2.2
Unknown (unspecified)	7	2.0	100	27.8
Total	360	100.0	360	100.0

TABLE 47

BLOOD DRAWING 3: TYPES OF NON-PRESCRIPTION MEDICATIONS USED
(QUESTION #7)

	Medication 1		Medication 2	
Non-prescription Medication	Frequency	% of Total	Frequency	% of Total
None	250	69.5	250	69.5
Tranquilizers	1	0.3	0	0
Stimulants	0	0	0	0
Antibiotics	1	0.3	0	0
Analgesics	89	24.7	1	0.3
Decongestants	6	1.7	3	0.8
Other	10	2.8	8	2.2
Unknown	3	0.7	98	27.2
Total	360	100.0	360	100.0

TABLE 48

BLOOD DRAWING 3: NUMBER OF STUDENTS WITH INCOMPLETE SCORING BY SEX FOR EACH OF THE EIGHT STUDY GROUPS

Group	No Metaphases Scored	1-24 Metaphases Scored	25-99 Metaphases Scored	100 Metaphases Scored	Total	% of Total with ≥ 25 Metaphases
MALE						
Low-Low	12	4	22	20	58	72.4
Low-High	9	8	7	5	29	41.4
High-Low	7	1	2	10	20	60.0
High-High	6	7	9	6	28	53.6
Low-High-Low	1	0	0	2	3	66.7
Low-High-High	0	1	2	0	3	66.7
High-Low-Low	1	2	1	6	10	70.0
High-Low-High	4	1	3	0	8	37.5
Total	40	24	46	49	159	59.7
FEMALE						
Low-Low	13	3	22	17	55	70.9
Low-High	9	8	15	12	44	61.4
High-Low	4	3	10	11	28	75.0
High-High	11	7	11	16	45	60.0
Low-High-Low	0	0	1	1	2	100.0
Low-High-High	2	0	1	0	3	33.3
High-Low-Low	3	0	3	3	9	66.7
High-Low-High	3	3	3	2	11	45.4
Total	45	24	66	62	197	65.0

TABLE 49

BLOOD DRAWING 3: COMPARISON OF MEANS OF CHROMOSOME ABNORMALITIES (PER 100 CELLS) BY STUDY GROUP, SEX, AND INFLUENCE OF COVARIATES

Abnormality and Sex of Students	Low-Low		Low-High		High-Low		High-High	
	A	B	A	B	A	B	A	B
Number of Students								
Males	40	36	7	5	9	8	13	11
Females	38	35	17	15	16	14	20	20
Aberrations								
Males	3.8	3.81	5.00	6.20	2.22	2.50	5.69	6.54
Females	3.16	3.23	4.06	4.53	6.25	5.64	3.20	3.20
Abnormal Cells								
Males	2.05	2.06	1.90	2.47	1.07	1.21	2.03	2.29
Females	1.71*	1.73	2.10*	2.32	3.13*	2.84	1.55*	1.55
Chromatid Breaks								
Males	2.07	2.06*	3.62	4.87*	1.07	1.21*	2.47	2.81*
Females	1.71*	1.73	2.23*	2.45	3.19*	2.91	1.60*	1.60
Chromatid Gaps								
Males	3.44**	3.40**	3.27**	4.18**	2.04**	2.13**	5.17*	5.81**
Females	2.77	2.87	3.48	3.68	3.28	3.11	3.39	3.39
Isochromatid Breaks								
Males	0.75	0.8[illegible]	1.57	2.00	0.22	0.25	0.68	0.80
Females	0.66	0.64	0.64	0.72	1.28	0.80	0.33	0.33
Isochromatid Gaps								
Males	0.05	0.06*	0.57	0.80*	0.33	0.38*	0.08	0.10*
Females	0.05	0.06*	0.14	0.16*	0.12	0*	0.42	0.42*
Polyradials								
Males	0.03	0.03	0	0	0	0	0.13	0.15
Females	0	0	0.06	0.07	0	0	0	0
Stable Changes								
Males	0.02	0**	0.29	0.40**	0	0**	0.15	0.18**
Females	0.06	0.06	0	0	0.06	0.07	0.05	0.05
Terminal Blebs								
Males	0.02	0.03	0	0	0	0	0	0
Females	0	0	0	0	0	0	0.06	0.07

A columns = all subjects tested
B columns = subjects without covariate exposure
* = .05< P≤ .10 as computed by F statistic
** = P ≤ .05 as computed by F statistic

TABLE 50

BLOOD DRAWING 3 - BLOOD DRAWING 1: COMPARISON OF MEANS OF CHROMOSOME ABNORMALITIES (PER 100 CELLS) BY STUDY GROUP SEX AND INFLUENCE OF COVARIATES

Abnormality and Sex of Students	Low-Low		Low-High		High-Low		High-High	
	A	B	A	B	A	B	A	B
Number of Students								
Males	39	32	7	4	9	8	13	8
Females	40	25	17	12	17	13	20	15
Aberrations								
Males	0.54	0.63	1.14	3.50	-0.89	-0.63	2.38	1.25
Females	0.75	0.68**	-1.12	-2.33**	2.24	3.92**	0.90	0.93**
Abnormal Cells								
Males	0.53	0.60	0.30	1.06	-0.04	0.21	0.79	0.97
Females	0.57	0.47*	0.01	-0.31*	1.30	1.90*	0.35	0.40*
Chromatid Breaks								
Males	0.46	0.54	1.59	3.31	-0.15	0.08	0.85	0.56
Females	0.45	0.36*	-0.05	-0.39*	1.24	1.90*	0.45	0.54*
Chromatid Gaps								
Males	1.40	1.58	0.99	1.81	0.26	0.63	2.33	2.31
Females	0.38	0.48	0.43	-0.09	0.38	0.37	1.84	1.67
Isochromatid Breaks								
Males	0.33	0.44	1.00	1.75	0	0	0.37	0.23
Females	0.37	0.54	0.28	0.49	0.59	0.51	0.08	0.11
Isochromatid Gaps								
Males	-0.20**	-0.19**	0.57**	1.00**	0.22**	0.25**	0.08**	0.14**
Females	-0.08*	0	0.08*	0.20	0.06*	0	0.37*	0.12
Polyradials								
Males	0.03	0.04	0	0	0	0	0.13	0.21
Females	-0.02	0	0	0	-0.06	0	0	0
Stable Changes								
Males	-0.10	-0.13	0	0	0	0	0	-0.13
Females	0.06	0.05	0	0	0.06	0.08	0.05	0.07
Terminal Blebs								
Males	-0.03	-0.03	0	0	0	0	-0.16	-0.27
Females	-0.08	-0.08	-0.24	-0.17	-0.18	-0.08	-0.08	-0.13

A columns = all subjects tested
B columns = subjects without covariate exposure
* = .05< P≤ .10 as computed by F statistic
** = P ≤ .05 as computed by F statistic

TABLE 51

BLOOD DRAWING 3: COMPARISON OF GROUP MEANS PER 100 CELLS FOR STABLE CHANGES OF MALE AND FEMALE STUDENTS FOR EACH OF THE FOUR STUDY GROUPS ACCORDING TO THE 15-DAY, 3-DAY, AND 1-DAY CRITERIA APPLIED TO THE LOW-TO-HIGH SMOG GROUP

15-Day	N		Males %	N		Females %
Low-Low	40		0.02	38		0.06
Low-High	7		0.29	17		0
High-Low	9		0	16		0.06
High-High	13		0.15	20		0.05
F Statistic	--	1.82	--	--	0.32	--
Level of Significance	--	0.15	--	--	0.81	--
3-Day						
Low-Low	40		0.02	38		0.06
Low-High	3		0	9		0
High-Low	9		0	16		0.06
High-High	13		0.15	20		0.05
F Statistic	--	1.48	--	--	0.17	--
Level of Significance	--	0.23	--	--	0.92	--
1-Day						
Low-Low	40		0.02	38		0.06
Low-High	2		0	5		0
High-Low	9		0	16		0.06
High-High	13		0.15	20		0.05
F Statistic	--	1.44	--	--	0.10	--
Level of Significance	--	0.24	--	--	0.96	--

CHAPTER 6

STATISTICAL ANALYSES FOR BLOOD DRAWING FOUR

DESCRIPTIVE CHARACTERISTICS OF STUDENTS

Blood was drawn from 320 students during the fourth blood sampling period. The dates of this blood sampling were September 4, 1977 to October 20, 1977 at the La Sierra campus of Loma Linda University and September 7, 1977 to October 13, 1977 at the Pacific Union College campus. The questionnaire used is found in Appendix E.

Table 52, page 83, portrays the number of students by sex in each study design group which had at least 25 metaphases scored by the Utah laboratory. The numbers of students in sex-specific study design groups were insufficient for statistical analyses.

QUALITY OF SLIDES

During the scoring of the third set of blood samples the Utah laboratory technicians detected what they felt was some deterioration in the quality of the slides. Because of this difficulty and due to the fact that they were already considerably behind schedule in completing the third set of slides, Loma Linda suggested that they abandon, for the time being, scoring the third set and concentrate their efforts on the fouth blood sample slides. This was done for two reasons; (1) Utah had already received the fourth set, and (2) it was felt that the fourth set, which completed a full year's cycle of blood sampling, might be more useful in determining any toxic effects of exposure to photochemical air pollution.

However, Utah also felt that the scoring quality of the fourth set of slides had likewise deteriorated. In addition, the increased time which Utah felt was necessary to score the slides in sets three and four had completely exhausted all the funds budgeted for this phase of the study.

It is of interest to note that in carrying out the inter-laboratory validity sub-study (Chapter 2, page 26) the Denver laboratory did not experience this same difficulty in scoring their ten percent random sample of slides from both blood sample sets three and four.

In order to determine possible reasons for the difficulty experienced by Utah in scoring the slides, a meeting was held at Loma Linda. Those who discussed the situation were cytogeneticists Willard Centerwall (Loma

Linda), Herbert Lubs (Denver), Paul Ing (Denver), and Randy Moon (Utah). Utah Biomedical Test Laboratory's cytogenetic technicians who had participated in the entire study were also present. Utah's main contention was that the quality of the slides was not as good as those which they had previously scored in the Unviersity of Southern California study of Scott and Burkart (9). The group concluded that the primary reason for Utah's difficulty in scoring slides was the inadequate quality (resolution, etc.) of their microscopes (as compared with those used by Denver). In addition, the Utah technicians were not familiar in scoring chromosomes which appeared to be banded.

CHEMICAL TOXICITY

During the culturing of the white blood cells from the fourth blood drawing at the Loma Linda laboratory, the technicians noted some difficulty securing the expected number of metaphases. Since the culture media being used for the fourth set was from the same batch as that used for the third set (which had been kept in a freezer), there was no advance warning of this problem. Willard Centerwall immediately had the Loma Linda laboratory recheck all culturing media and procedures. A number of new samples were tested and everything appeared adequate to continue with the study. Fresh blood samples were obtained from the students on each campus whose previous cell cultures lacked sufficient metaphases for scoring. Even though fewer metaphases than normal were harvested it was felt that there were sufficient numbers for scoring the minimum of one hundred per student. A step by step and chemical by chemical retropsective analysis of the possible reasons for the toxicity observed in the cell cultures suggested that the problem resulted from defective (higher strength) Bacto Phytohemagglutinin P (Difco). Several other genetic laboratories in the area experienced a similar effect at about the same time.

As a statistical check on the possibility of chemical toxicity in the fourth blood drawing, individual analyses of each laboratory cell culture batch were conducted. Of particular concern was the percentage of students with polyradial rates in excess of 1 percent. This criterion was suggested by Herbert Lubs as being a threshold above which one might suspect cytogenetic artifacts to be the result of chemical toxicity in the culturing media rather than in vivo effects. The results of this analysis are shown in Table 53, page 84. The Denver lab noticed no polyradials in their validity sub-sample of 10 percent of all slides, except for batches 28 and 29 where they detected 7 out of 11 students which they scored in batch 28 as having polyradials and 5 out of the 17 in batch 29. After discussing these results with Herbert Lubs and Paul Ing, it was decided that there was a definite possibility that there was some chemical toxicity in batches 28 and 29 of the fourth blood drawing.

Because of the possibility of chemical toxicity and because the number of students in each study design group by sex for which slides had been scored was too small to provide stable estimates (Table 52, page 83), attempts to conduct statistical analyses of the fourth blood drawing were abandoned.

TABLE 52

BLOOD DRAWING 4: NUMBER OF STUDENTS WITH INCOMPLETE SCORING BY SEX FOR EACH OF THE EIGHT STUDY GROUPS

Group	No Metaphases Scored	1-24 Metaphases Scored	25-99 Metaphases Scored	100 Metaphases Scored	Total	% of Total with ≥ 25 Metaphases
MALE						
Low-Low	29	0	1	12	42	30.9
Low-High	24	0	1	3	28	14.3
High-Low	13	0	0	2	15	13.3
High-High	27	0	1	1	29	6.9
Low-High-Low	1	0	0	1	2	50.0
Low-High-High	3	0	0	0	3	0
High-Low-Low	8	0	0	1	9	11.1
High-Low-High	7	0	0	2	9	22.2
Total	112	0	3	22	137	18.2
FEMALE						
Low-Low	34	0	5	9	48	29.2
Low-High	35	0	2	4	41	14.6
High-Low	15	0	1	7	23	34.8
High-High	34	0	4	7	45	24.4
Low-High-Low	1	0	0	1	2	50.0
Low-High-High	3	0	0	0	3	0
High-Low-Low	10	0	0	0	10	0
High-Low-High	8	0	1	1	10	20.0
Total	140	0	13	29	182	23.1

TABLE 53

PERCENTAGE OF STUDENT SLIDE SETS WITH ONE OR MORE POLYRADIALS BY LABORATORY BATCH AS SCORED BY THE UTAH LABORATORY				
BLOOD DRAWING NUMBER	LABORATORY BATCH NUMBER	NUMBER WITH ≥ 1 POLYRADIAL	TOTAL NUMBER	PERCENT
1	1	0	1	0
	2	0	49	0
	3	0	60	0
	4	0	60	0
	5	1	35	2.9
	6	0	47	0
	7	0	50	0
	8	0	42	0
	9	0	34	0
	10	0	25	0
2	11	0	49	0
	12	0	50	0
	13	0	60	0
	14	0	45	0
	15	0	40	0
	16	0	49	0
	17	0	53	0
	18	0	47	0
	19	0	9	0
3	20	0	39	0
	21	0	27	0
	22	1	10	10.0
	23	0	17	0
	24	0	19	0
	25	0	16	0
	26	0	24	0
	27	0	1	0
4	28*	4	26	15.4
	29	2	35	5.7
	30	0	6	0
	31	0	2	0
	32	0	1	0

*Batch 28 consists of 13 small sub-batches

DISCUSSION

This study was originally designed to validate and extend the observations made by Scott and Burkart (9) in their study of students attending the University of Southern California (USC) in the South Coast Air Basin of southern California. To aid in the comparison between the results of the two studies the same laboratory, Utah Biomedical Test Laboratory, was employed in the scoring of the chromosome spreads.

Our study design differed from Scott and Burkart (9) in two ways. First the students were carefully pre-screened for self-imposed and community exposure to chemicals which potentially could result in similar chromosome anomalies. Secondly, the students attended two different colleges, one in a high photochemical smog area and one in a low photochemical smog area. This last difference allowed for comparison of additional exposure groups, e.g. those with no significant previous exposure to photochemical smog remaining in such an environment or entering a high smog area during the study period.

While it was expected that cytogenetic analyses of the peripheral lymphocytes would be made over an entire year, with four blood sampling periods corresponding to the varying air pollution cycles of the region, several events prevented us from reaching this goal. These included the change in technical personnel at Utah Biomedical Test Laboratory, technician unfamiliarity with scoring banded chromosomes, and laboratory toxicity in blood cultures during the fourth blood sampling. It is for these reasons that the statistical analyses primarily focuses on the first three blood sampling periods.

In general, it was felt there were no significant differences between the four study groups at each blood sampling period or in temporal changes between the groups at different blood sampling periods.

An exception of statistical significance was the occurrence of stable changes observed during the initial blood sampling period. When the male low-to-high group was compared to the other three groups, by a decreasing time interval (15-day; 3-day; 1-day) between arrival on campus and blood sampling, the incidence of stable changes became statistically significantly elevated. Increased differences, while not as statistically significant were also noted for females in the same group. These elevated rates had dropped off by the second blood sampling two months later. The small sample size cautions one against concluding too much from the statistically significant differences. After considering the individual ozone exposures of the students, there appeared to be a lack of conclusive evidence that exposure to ozone produced the stable changes in the peripheral lymphocyte chromosomes, although there is some possibility that there may be a shock effect for some students; that is, exposure to ozone or some other environmental factor, may affect the peripheral lymphocyte chromosomes in a very short period of time, e.g. less than 24 hours. Such a possibility has been indicated (3, 7).

We are confident that our data do not support the hypothesis that living in the South Coast Air Basin of southern California with its high photochemical air pollution significantly affects the chromosome integrity of the peripheral blood lymphocytes. Nor is there evidence that those living in areas of little or no photochemical air pollution have statistically significant fewer chromosome abnormalities.

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APPENDIX A

Exhibit 1

Pre-Enrollment Student Questionnaires
For Selection of Study Groups

Confidential

1. Sex (circle one) M F

2. Age __________

3. Date of Birth ___ ___ ___

4. Of what ethnic origin do you consider yourself?

   ☐ American-Indian
   ☐ Black
   ☐ Caucasian
   ☐ Mexican-American
   ☐ Oriental
   ☐ Other ______________________

5. Year in college ________________

6. Are you planning to attend this campus both this year and next year?

   ☐ Yes
   ☐ No
   ☐ Will be at different campus next year (please specify) __________________
   ☐ Not sure

7. Planned college residence (address or dormitory) ____________________________

   ________________________________ Phone # if known ______________________

8. Phone # where you can be reached between now and the beginning of school ____________________

9. Where have you lived during the past five years? Start with your current residence and work backwards. (For large cities, please give the section of the city. If the town you have lived in is so small that it does not have a post office, give the nearest town with a post office.

TOWN	STATE	Moved to town: MONTH	 YEAR	Moved from town: MONTH	 YEAR

Please answer the questions on the back side
(continued)

APPENDIX A (continued)

Exhibit 1 (continued)

Medical History

1. Have you ever been employed in an industry which manufactured, processed, or handled chemicals, such as solvents, rubber, insecticides, or explosives? If yes, please indicate when and list the chemicals used. ______________________________
____ Yes ☐ No ☐

2. Are you using any perscription drugs? If yes, what drugs?
____ Yes ☐ No ☐

3. Are you using any non-perscription drugs, such as aspirin, cough syrup, No-Doz, Sleep-eze, etc.? If yes, what drug(s)?
____ Yes ☐ No ☐

4. Have you been treated in the last year, by drugs for a medical problem for a period of more than four weeks? Yes ☐ No ☐

5. Have you ever been treated by X-radiation, other than for diagnostic purposes, in a radiotherapy department for a medical problem? Yes ☐ No ☐

6. Have you regularly smoked cigarettes during the past year? Yes ☐ No ☐

7. Have you used drugs, such as marijuana, cocaine, LSD, etc. more than once or twice during the past year? Yes ☐ No ☐

8. Have you ever received treatment for tuberculosis? Yes ☐ No ☐

9. Have you ever received treatment for a thyroid gland problem? Yes ☐ No ☐

(continued)

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DROPPED HEAD BEGIN SECTIONS HERE

Exhibit 2

INSTRUCTIONS FOR TELEPHONE INTERVIEWERS

The following is a list of instructions that you must follow to obtain the accurate information we need for the Environmental Protection Agency Student Study. Please read through all of the instructions very carefully before you make any telephone calls.

If you have any questions regarding this telephone work, please contact me. My number is 796-7311, Ext. 3726. If you need to reach me during your working period, you may call me at 794-2262.

You will be given an assignment sheet with the names and telephone numbers of the people you are to call. Be sure not to lose this sheet. You are to make these calls between 5:00 p.m. and 9:00 p.m. starting Monday and continuing through Thursday of this week. If your assignment has not been finished by Thursday, then continue next Monday until finished. All phone calls must be completed by Thursday, September 2. If a person is not home when you call, be sure to call back until you are able to reach them.

It is most important that you speak very clearly and use a pleasant voice. All questions must be answered. If a person refuses to answer a question, write "refused" by the question they do not wish to answer. Be very polite; never leave an angry or hostile impression.

You will be assigned an interviewer number. It is most important that you put this on the information form as well as the person's research number which will be found beside the person's name on your assignment sheet. Be sure the correct research number and interviewer number are on the telephone form.

When you are ready to make your calls, follow this procedure. Take the telephone information form, which you will be given a supply of, and clip it to a questionnaire. On this paper, write your interviewer number, the person's research number and telephone number, and the date called. After you have completed the call, indicate on this same sheet of paper the response. Such as, if the person was home and you filled out the telephone questionnaire, say "telephone questionnaire completed". If the person refused to participate in the study, say "does not wish to participate in study". If the person is sending in the questionnaire they received, say "mailing questionnaire in", or if they have already mailed it in, say "have mailed questionnaire in". If the person is not home, indicate on the form "call back" and set this questionnaire aside and call back later.

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3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

Exhibit 2 (continued)

The following introduction will be used. Please read it over carefully.

"Hello, I am __________ from the Survey Research Service of Loma Linda University, calling in regards to the study of possible effects of air pollution on health. We need volunteers for this study so we are calling on you. Did you receive the questionnaire that we sent to you?" (If they say yes, then ask them if they have filled it out and returned it to us. If they have, ask them for the approximate date it was mailed, indicate that on your information sheet, and thank them for their cooperation. If they say they have received the questionnaire but do not wish to participate, indicate this information on the information sheet and thank them. If they say they have not had time to fill it out, ask them if they would like to give you the information over the phone. Then if they wish to do this, continue on with the questions on the questionnaire. If they say they will fill out the questionnaire they received, ask them to do it immediately and mail it, as we need to have them this week. If they say they have not received the questionnaire, then proceed with the following explanation.)

"This study is being sponsored by the Environmental Protection Agency and focuses on characteristics of the blood. They are placing a monitoring station right at La Sierra Campus or Pacific Union College (use the one that applies) this year. Students who participate in this study will need to answer a few questions regarding medical history and fill out a questionnaire when they arrive on campus and have their blood drawn four times, once each quarter throughout the coming year. Total amount of blood drawn will be very small, and each person who participates will be given $40.00 in appreciation for their participation. Do you think you might be interested in participating in the study?" (If their answer is yes, then ask them if they would be willing to answer the questions on the phone; and, if they agree, then proceed with the questionnaire. If they say they do not wish to participate in the study, then thank them.)

When you are finished with the calls that have been assigned to you, please bring your assignment sheet, questionnaires and telephone information sheets to me. Keep track of your time on the green time card provided for this purpose. When you receive your phone bill, bring it to me and I will process it for payment on the calls you have made for Survey Research Service.

(continued)

Exhibit 2 (continued)

E. P. A. S. A. P. S.

TELEPHONE INFORMATION FORM

INTERVIEWER # ______________

RESEARCH # ________________

TELEPHONE # _______________

DATE CALLED _______________

RESPONSE

(continued)

APPENDIX A (continued)

Exhibit 3

Research No. $\underset{1}{\_}\ \underset{2}{\_}\ \underset{3}{\_}\ \underset{4}{\_}$

Form No. $\underset{56}{\underline{02}}$

Interviewer No. $\underset{8}{\_}\ \underset{9}{\_}$

PRE-ENROLLMENT STUDENT TELEPHONE QUESTIONNAIRE FOR SELECTION OF STUDY GROUPS

Section A.

1. Have you ever been employed in an industry which manufactured, processed, or handled chemicals, such as solvents, rubber, insecticides, or explosives? If yes, please indicate when and list the chemicals used. — ^{1}[ ] No ^{2}[ ] Yes

Dates	Chemicals
____________	____________
____________	____________
____________	____________

2. Are you using any perscription drugs? List drugs. — ^{1}[ ] No ^{2}[ ] Yes

____

3. Are you using any non-perscription drugs, such as aspirin, cough syrup, No-Doz, Sleep-eze, etc.? List drugs. — ^{1}[ ] No ^{2}[ ] Yes

____

4. Have you been treated in the last year, by drugs for a medical problem for a period of more than four weeks? List drugs. — ^{1}[ ] No ^{2}[ ] Yes

____

Section B.

NOW I WILL ASK YOU FOUR QUESTIONS. I DO NOT NEED TO KNOW THE ANSWER FOR EACH INDIVIDUAL QUESTION. BUT AFTER ASKING ALL 4 QUESTIONS I WILL NEED TO KNOW IF YOUR ANSWER WAS YES TO ANY ONE OF THEM.

1. Have you ever been treated by X-radiation for a medical problem other than for screening purposes such as chest and dental X-rays?
2. Have you regularly smoked cigarettes during the past year?
3. Have you used drugs, such as marijuana, cocaine, LSD, etc., more than once or twice during the past year?
4. Have you ever received treatment for tuberculosis?
5. Was your answer "YES" to any of these questions?

   [ ] No - Please continue with questions on back.

   [ ] Yes - Ask following 2 questions and end interview.

   Age ______

   Year in college __________

Thank-you very much. We will let you know if you are selected for the study.

(continued)

APPENDIX A (continued)

Exhibit 3 (continued)

Section C.

1. Sex

   ^{1}[ ] M

   ^{2}[ ] F

2. _ _ Age

3. Of what ethnic origin do you consider yourself?

   ^{1}[ ] American-Indian

   ^{2}[ ] Black

   ^{3}[ ] Caucasian

   ^{4}[ ] Mexican-American

   ^{5}[ ] Oriental

   ^{6}[ ] Other ____________________

4. _____ Year in college

5. Are you planning to attend this campus both this year and next year?

   ^{1}[ ] Yes

   ^{2}[ ] No

   ^{3}[ ] Will be at different campus next year (please specify) ________________

   ^{4}[ ] Not sure

6. Planned college residence (address or dormitory) ________________________

   ______________________________ Phone # if known ________________

7. Phone # where you can be reached between now and the beginning of school

   ____

8. Where have you lived during the past five years? Start with your current residence and work backwards. (For large cities, please give the section of the city. If the town you have lived in is so small that it does not have a post office, give the nearest town with a post office.

		Moved to town:		Moved from town:	
TOWN	STATE	MONTH	YEAR	MONTH	YEAR
________________________		_____	_____	_____	_____
________________________		_____	_____	_____	_____
________________________		_____	_____	_____	_____
________________________		_____	_____	_____	_____

Thank you very much. We will let you know if you are selected for the study.

(continued)

Exhibit 4

Dear Student:

I extend my congratulations on your being accepted for study at La Sierra campus of Loma Linda University. The college administration has granted our research team permission to solicit your cooperation in a study of potentially important medical significance. This is an opportunity for young people to become involved in and contribute to the advance of medical science.

Briefly, the study, which is being sponsored by the United States Environmental Protection Agency (EPA), will seek to determine whether or not photochemical air pollution affects the health of college students. An elaborate and sensitive air monitoring station will be set up on campus by the EPA. We are particularly interested in potential genetic changes which would have a tremendous influence on generations to come. However, let me assure you that there is no known risk to your health if you study or live in a smoggy area.

The Schools of Health and Medicine at Loma Linda University are responsible for conducting this two year study. During this period you will be asked to visit the student health service four times to provide a blood sample (the total amount of all four will be only a few ounces) and answer a few questions. All questionnaires and the results of blood analyses will be kept ABSOLUTELY CONFIDENTIAL. No college official will receive a report and the results will be published only as group summaries. 200 students will make up the study group on your campus. If findings of health significance are found only you will be notified--leaving the decision for medical or other assistance your responsibility.

Should you be selected as part of the study group, you will receive a small financial remuneration ($40.00 for the complete study; $10.00 at the time of each required visit to the student health service) as our appreciation for your assistance and cooperation. Should you so desire you may drop out of the study at any time. However, this would considerably weaken its value.

Please complete the enclosed questionnaire (which focuses its attention on those environmental agents known to affect chromosomes) and return it in the postage-paid envelope provided. If you are not sure that you will be attending college this fall or you do not wish to participate please mark the enclosed card accordingly and return it immediately.

Should you have any questions regarding this study or your participation, please write or call.

Sincerely,

Allan R. Magie

Allan R. Magie, Ph.D., M.P.H.
Principal Investigator and Associate Professor
of Environmental and Tropical Health

ARM:cll

(continued)

APPENDIX A (continued)

Exhibit 5

Dear Student:

I extend my congratulations on your being accepted for study at Pacific Union College. The college administration has granted our research team permission to solicit your cooperation in a study of potentially important medical significance. This is an opportunity for young people to become involved in and contribute to the advance of medical science.

Briefly, the study, which is being sponsored by the United States Environmental Protection Agency (EPA), will seek to determine whether or not photochemical air pollution affects the health of college students. An elaborate and sensitive air monitoring station will be set up on campus by the EPA. We are particularly interested in potential genetic changes which would have a tremendous influence on generations to come. However, let me assure you that there is no known risk to your health if you study or live in a smoggy area.

The Schools of Health and Medicine at Loma Linda University are responsible for conducting this two-year study. During this period you will be asked to visit the student health service four times to provide a blood sample (the total amount of all four will be only a few ounces) and answer a few questions. All questionnaires and the results of blood analyses will be kept ABSOLUTELY CONFIDENTIAL. No college official will receive a report and the results will be published only as group summaries. Two hundred students will make up the study group on your campus. If findings of health significance are found, only you will be notified--leaving the decision for medical or other assistance your responsibility.

Should you be selected as part of the study group, you will receive a small financial remuneration ($40.00 for the complete study; $10.00 at the time of each required visit to the student health service) as our appreciation for your assistance and cooperation. Should you so desire, you may drop out of the study at any time. However, this would considerably weaken its value.

Please complete the enclosed questionnaire (which focuses its attention on those environmental agents known to affect chromosomes) and return it in the postage-paid envelope provided. If you are not sure that you will be attending college this fall or you do not wish to participate, please mark the enclosed card accordingly and return it immediately.

Should you have any questions regarding this study or your participation, please write or call.

Sincerely,

Allan R. Magie

Allan R. Magie, Ph.D., M.P.H.
Principal Investigator and Associate Professor
of Environmental and Tropical Health

(continued)

Exhibit 6

REFUSAL FORM

_____ I am not sure that I will be attending college this year.

_____ I do not wish to participate in this study.

Name ______________________________________

Address ____________________________________

____

APPENDIX B

CLASSIFICATION OF CITIES FROM WHICH STUDENTS CAME PRIOR TO BLOOD DRAWING ONE ACCORDING TO AMBIENT AIR POLLUTION

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1005	2	Anchorage	L	Alaska	99504
1006	4	Colton	H	Calif.	92324
1007	4	Supulveda	I	Calif.	91343
1008	4	Los Angeles	H	Calif.	91776
1011	4	Hacienda Hghts.	H	Calif.	91745
1014	2	Phoenix	L	Arizona	85301
1016	8	Riverside	I	Calif.	92505
1017	2	Bishop	L	Calif.	93513
1018	4	Burbank	I	Calif.	91344
1019	6	Riverside	H	Calif.	92505
1024	2	Auburn	L	Wash.	98002
1026	6	San Bernardino	H	Calif.	91730
1027	2	Loveland	L	Colo.	80537
1028	2	Ridgecrest	L	Calif.	93555
1029	4	Riverside	H	Calif.	92505
1030	4	Riverside	H	Calif.	92505
1032	4	Hacienda Hghts.	H	Calif.	91745
1033	2	Seaside	L	Calif.	93955
1038	2	Santa Barbara	L	Calif.	93460

* see chapter 1, page 9

+ H = high photochemical air pollution
L = low photochemical air pollution
S = low photochemical air pollution but high SO_2
I = Intermediate photochemical air pollution

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1039	4	Los Angeles	H	Calif.	90713
1041	4	Pacoima	I	Calif.	91331
1047	4	San Fernando	I	Calif.	91351
1048	4	Loma Linda	H	Calif.	92354
1052	2	Long Beach	I	Calif.	90805
1053	4	Riverside	H	Calif.	92505
1056	4	Cypress	H	Calif.	90630
1057	2	Lakeside	L	Calif.	92040
1059	4	Loma Linda	H	Calif.	92354
1060	2	Sacramento	L	Calif.	95351
1066	2	Santa Barbara	L	Calif.	93463
1068	2	Blue Jay	I	Calif.	92317
1069	4	Hollywood	H	Calif.	90004
1070	4	Whittier	H	Calif.	90605
1071	2	Escondido	L	Calif.	92026
1072	8	Los Angeles	H	Calif.	90631
1073	2	San Diego	L	Calif.	92041
1074	2	San Diego	L	Calif.	92041
1076	2	Portland	H	Oregon	97236
1077	2	Kingman	L	Arizona	86430
1079	2	Boulder	L	Colo.	80302
1080	2	Palos Verdes	I	Calif.	90274
1082	2	Irvine	I	Calif.	92714
1083	4	La Puente	H	Calif.	91744
1084	4	Norwalk	H	Calif.	90650
1087	4	San Bernardino	H	Calif.	92407
1088	2	Tucson	L	Arizona	85704
1090	4	Glendale	H	Calif.	91209
1091	8	Alhambra	H	Calif.	91801

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1093	2	Palos Verdes	I	Calif.	90274
1094	4	Montclair	H	Calif.	91763
1097	2	Torrance	I	Calif.	90510
1104	4	Riverside	H	Calif.	92505
1105	4	Redlands	H	Calif.	92373
1108	4	Duarte	H	Calif.	91010
1114	4	Riverside	H	Calif.	92504
1117	8	La Sierra	H	Calif.	92505
1130	4	Sun Valley	H	Calif.	91352
1135	6	Angwin	L	Calif.	94508
1138	4	Whittier	H	Calif.	90602
1139	2	Portland	I	Oregon	97236
1140	6	Redlands	H	Calif.	92373
1142	2	Cortez	L	Colo.	81321
1144	4	San Fernando	I	Calif.	93065
1153	2	Escondido	L	Calif.	92075
1157	4	Chatsworth	I	Calif.	91311
1160	4	Woodland Hills	I	Calif.	91364
1164	2	San Diego	L	Calif.	92139
1167	4	Loma Linda	H	Calif.	92354
1168	4	Adelanto	I	Calif.	92301
1172	4	Garden Grove	H	Calif.	92640
1174	4	La Habra	H	Calif.	90631
1176	2	Phoenix	L	Arizona	85033
1182	2	Escondido	L	Calif.	92027
1184	2	Santa Maria	L	Calif.	93454
1185	2	Santa Barbara	L	Calif.	93013
1186	2	Dayton	S	Ohio	45400

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1187	2	Fresno	I	Calif.	93230
1196	4	Los Angeles	H	Calif.	92305
1199	4	Colton	H	Calif.	92324
1201	6	Santa Cruz	L	Calif.	95060
1204	2	Fresno	I	Calif.	93277
1206	4	Fontana	H	Calif.	92335
1207	2	Phoenix	L	Arizona	85302
1210	2	San Diego	L	Calif.	92111
1214	4	Whittier	H	Calif.	90603
1219	2	Capitola	L	Calif.	95010
1220	8	Riverside	H	Calif.	92505
1221	2	Phoenix	L	Arizona	85031
1223	4	El Monte	H	Calif.	91731
1231	8	Loma Linda	H	Calif.	92354
1234	4	Los Angeles	H	Calif.	90280
1235	2	San Jose	L	Calif.	95026
1236	4	La Sierra	H	Calif.	92505
1240	8	La Sierra	H	Calif.	92505
1242	2	Kalamazoo	L	Mich.	49034
1246	8	Cypress	H	Calif.	90630
1248	8	Riverside	H	Calif.	92505
1249	4	La Sierra	H	Calif.	92505
1250	2	Sacramento	L	Calif.	95307
1253	2	San Diego	L	Calif.	92024
1255	4	Bellflower	I	Calif.	90706
1256	8	Redlands	H	Calif.	92373
1258	4	San Bernardino	H	Calif.	92405
1259	4	Arcadia	H	Calif.	91006
1260	8	Palm Springs	H	Calif.	92276

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1262	2	Walla Walla	L	Wash.	99362
1263	8	La Sierra	H	Calif.	92505
1264	2	Saugus	I	Calif.	91350
1265	2	La Mesa	L	Calif.	92041
1270	2	Escondido	L	Calif.	92025
1272	8	Riverside	H	Calif.	92505
1273	4	La Sierra	H	Calif.	92505
1276	4	Los Angeles	H	Calif.	90041
1283	2	Rolling Hills	L	Calif.	90274
1285	2	El Cajon	L	Calif.	92021
1287	4	Rialto	H	Calif.	92376
1290	4	Simi Valley	I	Calif.	93065
1291	4	Cerritos	H	Calif.	90701
1296	2	San Diego	L	Calif.	92050
1299	4	Corona	H	Calif.	91720
1301	4	Culver City	H	Calif.	90230
1304	2	San Diego	L	Calif.	92020
1307	4	Glendale	H	Calif.	91202
1310	2	National City	L	Calif.	92050
1311	2	Bakersfield	L	Calif.	93308
1317	4	Redlands	H	Calif.	92373
1319	4	Lakewood	H	Calif.	90713
1320	2	Watsonville	L	Calif.	95076
1321	4	Covina	H	Calif.	91724
1328	4	Mira Loma	H	Calif.	91752
1329	4	Sylmar	I	Calif.	91342
1333	2	Solvang	L	Calif.	93463
1336	4	La Sierra	H	Calif.	92505
1337	8	Los Angeles	H	Calif.	91214

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1340	4	Glendale	H	Calif.	91206
1345	2	Escondido	L	Calif.	92025
1346	4	Clairmont	H	Calif.	91711
1350	4	Riverside	H	Calif.	92505
1355	6	Angwin	L	Calif.	94508
1364	2	Angwin	L	Calif.	94508
1373	4	La Sierra	H	Calif.	92505
1379	2	Dayton	S	Ohio	45400
1407	4	La Sierra	H	Calif.	92505
1409	4	Simi Valley	I	Calif.	93005
1410	6	Angwin	L	Calif.	94508
1419	8	La Verne	H	Calif.	91750
1422	4	Hesperia	I	Calif.	92345
1426	2	Lancaster	I	Calif.	93534
1427	2	San Bernardino	I	Calif.	92405
1430	4	Northridge	I	Calif.	91324
1431	2	Laguna Beach	L	Calif.	92651
1433	4	La Sierra	H	Calif.	92505
1434	2	Red Deer	L	Alberta	CAN
1445	8	Fullerton	I	Calif.	92633
1463	2	Walla Walla	L	Wash.	99324
1481	2	Columbus	L	Ind.	47201
1484	4	Valencia	I	Calif.	91355
1486	2	Delta	L	Col.	81416
1490	8	Loma Linda	H	Calif.	92354
1491	4	San Bernardino	H	Calif.	92408
1492	2	Chicago	L	Illinois	49031
1506	2	Fallon	L	Nevada	89406
1509	4	La Habra	H	Calif.	90631

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION	STATE OF RESIDENCE	POSTAL ZIP CODE
1512	2	Lancaster	L	Mass	01523
1513	2	Beirut	L	Lebanon	
1514	2	Santa Barbara	L	Calif.	93013
1516	4	Yucaipa	H	Calif.	92339
1517	4	Yucaipa	H	Calif.	92339
1518	2	San Diego	L	Calif.	92139
1519	2	Escondido	L	Calif.	92027
1520	4	Glendale	H	Calif.	91206
1521	2	Lansing	L	Mich.	48867
1522	6	San Jose	L	Calif.	95140
1524	2	Chula Vista	L	Calif.	97010
1525	2	Jersey City	S	N.J.	07307
1526	2	Angwin	L	Calif.	94508
1528	4	Glendale	H	Calif.	91205
1529	2	Denver	L	Col.	80210
1530	2	Watsonville	L	Calif.	95076
1531	2	Lansing	L	Mich.	48900
1532	6	Collonges-Sous-Saleve	L	France	
1533	4	Chatsworth	I	Calif.	91311
1534	2	Denver	S	Colo.	80513
1535	2	Prescot	L	Arizona	86301
1536	4	Glendale	H	Calif.	91214
1537	2	Amman	L	Jordan	
1538	4	Loma Linda	H	Calif.	92354
1539	2	El Paso	S	Texas	79925
1540	4	Alhambra	H	Calif.	91803
1541	2	Fresno	L	Calif.	93654
1542	2	Santa Barbara	L	Calif.	93460
1543	2	Paso Robles	L	Calif.	93446

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
1544	2	San Diego	L	Calif.	92116
1545	8	La Sierra	H	Calif.	92505
1547	4	Downey	H	Calif.	90241
1548	4	San Fernando	H	Calif.	91325
1549	2	Roslyn	L	New York	11576
1550	2	Gimiligen	L	Switzerland	3006
1551	8	Glendale	H	Calif.	91208
1552	2	Angwin	L	Calif.	94508
1553	8	La Sierra	H	Calif.	92505
1554	4	Los Angeles	H	Calif.	90022
1555	4	Lynwood	H	Calif.	90761
1556	6	Escondido	L	Calif.	92025
2004	1	Angwin	L	Calif.	94508
2038	1	Milwaukee	S	Wisc.	53216
2072	1	Cortez	L	Colo.	81321
2086	1	Angwin	L	Calif.	94508
2087	1	Angwin	L	Calif.	94508
2094	1	Las Vegas	L	Nevada	89102
2096	1	Reno	L	Nevada	89447
2101	1	Reno	L	Nevada	89406
2104	1	Reno	L	Nevada	89503
2110	7	Los Angeles	H	Calif.	90065
2112	7	Los Angeles	H	Calif.	94508
2114	3	Lynwood	H	Calif.	90262
2116	7	Venice	H	Calif.	90262
2123	3	Norwalk	I	Calif.	90714
2124	3	Altadena	H	Calif.	91001
2125	5	Arcadia	L	Calif.	91006

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2127	3	La Canada	I	Calif.	91011
2128	3	La Canada	I	Calif.	91011
2129	3	La Canada	I	Calif.	91011
2130	7	Tujunga	H	Calif	91042
2131	3	Glendale	H	Calif.	91205
2132	3	Glendale	H	Calif.	91205
2134	3	Glendale	H	Calif.	91206
2140	3	Glendale	H	Calif.	91206
2141	3	Glendale	H	Calif.	91206
2142	5	Monterey Bay	L	Calif.	95076
2143	3	Glendale	H	Calif.	91206
2147	7	Glendale	H	Calif.	91208
2149	3	Newbury Park	I	Calif.	91320
2153	3	Northridge	I	Calif.	91325
2159	3	Van Nuys	H	Calif.	91401
2160	3	Van Nuys	H	Calif.	91406
2162	5	Santa Rosa	L	Calif.	95448
2165	3	Monterey Park	H	Calif.	91754
2168	3	Pomona	I	Calif.	91766
2173	1	San Diego	L	Calif.	92010
2185	1	El Cajon	L	Calif.	92021
2188	1	San Diego	L	Calif.	92050
2189	1	Angwin	L	Calif.	94508
2191	1	Romona	L	Calif.	92065
2196	1	Valley Center	L	Calif.	92082
2199	1	San Diego	L	Calif.	92114
2201	1	San Carlos	L	Calif.	92120
2204	1	National City	L	Calif.	92139

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2206	3	Banning	H	Calif.	92220
2209	3	Banning	H	Calif.	92220
2212	7	Colton	H	Calif.	92324
2215	7	Grand Terrace	H	Calif.	92324
2216	3	Grand Terrace	H	Calif.	92324
2217	1	Angwin	L	Calif.	94508
2219	3	Apple Valley	H	Calif.	92307
2220	3	Loma Linda	H	Calif.	92354
2221	3	Loma Linda	H	Calif.	92354
2222	3	Loma Linda	H	Calif.	92354
2223	3	Loma Linda	H	Calif.	92354
2228	7	Loma Linda	H	Calif.	92354
2229	3	Loma Linda	H	Calif.	92354
2233	7	Brush	L	Colo.	80723
2236	3	Loma Linda	H	Calif.	92354
2238	3	Loma Linda	H	Calif.	92354
2240	7	Loma Linda	H	Calif.	92354
2242	3	Loma Linda	H	Calif.	92354
2244	3	Redlands	H	Calif.	92373
2245	7	Redlands	H	Calif.	92373
2247	3	Redlands	H	Calif.	92373
2248	3	Redlands	H	Calif.	92373
2249	7	Redlands	H	Calif.	92373
2253	3	Redlands	H	Calif.	92373
2254	3	Redlands	H	Calif.	92373
2255	7	Redlands	H	Calif.	92373
2256	7	Redlands	H	Calif.	92373
2257	7	Redlands	H	Calif.	92373
2261	3	San Bernardino	H	Calif.	92404

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2274	3	La Sierra	H	Calif.	92505
2275	3	La Sierra	H	Calif.	92505
2277	3	Brea	H	Calif.	92621
2282	3	Anaheim	H	Calif.	92803
2297	1	Armona	I	Calif.	93202
2323	1	E. Bakersfield	L	Calif.	93305
2324	1	Bakersfield	L	Calif.	93308
2338	1	Cambria	L	Calif.	93428
2349	1	Clovis	L	Calif.	93612
2354	1	Madera	L	Calif.	93637
2372	1	Angwin	L	Calif.	94508
2373	1	Monterey	L	Calif.	95076
2381	1	Lodi	L	Calif.	95240
2387	1	Los Altos	L	Calif.	94022
2400	1	Mt. View	L	Calif.	94040
2409	1	Angwin	L	Calif.	94508
2416	1	Sunnyvale	L	Calif.	94087
2427	1	Angwin	L	Calif.	94508
2429	1	Oakland	L	Calif.	94605
2430	1	Angwin	L	Calif.	94508
2432	1	St. Joseph	L	Mich.	49085
2434	1	Angwin	L	Calif.	94508
2435	1	Angwin	L	Calif.	94508
2437	1	Angwin	L	Calif.	94508
2454	1	Angwin	L	Calif.	94508
2457	1	Angwin	L	Calif.	94508
2464	1	Angwin	L	Calif.	94508
2467	1	Calistoga	L	Calif.	94515

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2482	1	St. Helena	L	Calif.	94574
2483	1	Napa	L	Calif.	94558
2493	1	Napa	L	Calif.	94508
2505	1	Pope Valley	L	Calif.	94567
2506	1	Angwin	L	Calif.	94508
2516	1	Deer Park	L	Calif.	94576
2519	1	San Leandro	L	Calif.	94577
2521	1	San Leandro	L	Calif.	94578
2543	1	Novato	L	Calif.	94947
2545	1	San Francisco	L	Calif.	94952
2547	1	Petaluma	L	Calif.	94952
2549	1	Angwin	L	Calif.	94508
2550	1	Campbell	L	Calif.	95008
2551	1	San Jose	L	Calif.	95008
2555	1	Campbell	L	Calif.	95008
2557	1	Hurricane	L	Utah	84737
2558	1	San Jose	L	Calif.	95014
2568	1	Santa Cruz	L	Calif.	95000
2571	1	Santa Cruz	L	Calif.	95060
2578	1	Watsonville	L	Calif.	95076
2583	1	San Jose	L	Calif.	95110
2587	1	Angwin	L	Calif.	94508
2588	1	Angwin	L	Calif.	94508
2602	1	Stockton	L	Calif.	92504
2604	1	Lodi	L	Calif.	95240
2607	1	Lodi	L	Calif.	95240
2614	1	Lodi	L	Calif.	95240
2619	1	Manteca	L	Calif.	95336
2620	1	Angwin	L	Calif.	94508

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2638	1	Modesto	L	Calif.	95307
2643	1	Modesto	L	Calif.	95355
2645	1	Sonora	L	Calif.	95370
2646	1	San Jose	L	Calif.	95376
2650	1	Turlock	L	Calif.	95380
2664	1	Monterey Bay	L	Calif.	95076
2668	1	Healdsburg	L	Calif.	95448
2671	1	Healdsburg	L	Calif.	95448
2679	1	Santa Rosa	L	Calif.	95402
2683	1	Sonoma	L	Calif.	95476
2685	1	Ukiah	L	Calif.	95482
2688	1	Ukiah	L	Calif.	95482
2691	1	Angwin	L	Calif.	94508
2693	1	Eureka	L	Calif.	95501
2705	1	Auburn	L	Calif.	95603
2707	1	Auburn	L	Calif.	95603
2713	1	Carmichael	L	Calif.	95608
2714	1	Carmichael	L	Calif.	95608
2720	1	Fair Oaks	L	Calif.	95628
2721	1	Napa	L	Calif.	94515
2724	1	Fair Oaks	L	Calif.	95628
2725	1	Fair Oaks	L	Calif.	95628
2726	1	Fair Oaks	L	Calif.	95628
2727	1	Fair Oaks	L	Calif.	95628
2730	1	Orangevale	L	Calif.	95628
2733	1	Paradise	L	Calif.	95662
2734	1	Placerville	L	Calif.	95969
2738	1	Rancho Cordova	L	Calif.	95969
2741	1	Shingle Springs	L	Calif.	95670

(continued)

APPENDIX B (continued)

STUDENT I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2742	1	San Francisco	L	Calif.	94101
2744	1	W. Sacramento	L	Calif.	95691
2746	1	Sacramento	L	Calif.	95695
2749	1	Lovelock	L	Nevada	89419
2750	5	Rio Linda	I	Calif.	95673
2761	1	Sacramento	L	Calif.	95313
2775	5	Santa Rosa	L	Calif.	95401
2777	1	Grass Valley	L	Calif.	95945
2779	1	Grass Valley	L	Calif.	95945
2790	1	Paradise	L	Calif.	95969
2792	1	Yuba City	L	Calif.	95001
2794	1	Napa	L	Calif.	94558
2798	1	Healdsburg	L	Calif.	95448
2809	1	Susanville	L	Calif.	96130
2814	1	Hickam	L	Hawaii	96553
2818	1	Kailwa	L	Hawaii	96734
2840	1	Red Deer	L	Alberta	CAN
2850	1	Eugene	L	Oregon	97459
2852	1	Burn	L	Oregon	97501
2856	1	Ashland	L	Oregon	97520
2858	1	Lakeview	L	Oregon	97630
2859	1	Auburn	L	Wash.	98002
2860	1	Auburn	L	Wash.	98002
2863	1	Angwin	L	Calif.	94508
2868	1	Angwin	L	Calif.	94508
2875	7	Glendale	H	Calif.	91206
2896	3	Glendale	H	Calif.	91206
2897	7	Redlands	H	Calif.	92373
2899	3	Loma Linda	H	Calif.	92354

(continued)

APPENDIX B (continued)

STUDENTS I.D.	GROUP CLASSIFICATION*	NEAREST CITY TO LAST RESIDENCE	CITY CLASSIFICATION+	STATE OF RESIDENCE	POSTAL ZIP CODE
2900	3	Grand Terrace	H	Calif.	92324
2901	3	Glendale	H	Calif.	91206
2902	7	Redlands	H	Calif.	92373
2903	3	Redlands	H	Calif.	92373
2904	3	Glendale	H	Calif.	91206
2905	7	Yucaipa	H	Calif.	92399
2906	3	Glendale	H	Calif.	91206
2907	7	Riverside	H	Calif.	92503
2908	3	Loma Linda	H	Calif.	92354
2910	7	Glendale	H	Calif.	91205
2912	3	Glendale	H	Calif.	91203
2913	7	Glendale	H	Calif.	91206
2914	3	Loma Linda	H	Calif.	92354
2915	7	Los Angeles	H	Calif.	90201
2916	1	Monterey Bay	L	Calif.	95076
2917	1	Ashland	L	Oregon	97520
2935	1	Longbeach	I	Calif.	90807

* see chapter 1, page 9

+ H = high photochemical air pollution
L = low photochemical air pollution
S = low photochemical air pollution but high SO_2
I = intermediate photochemical air pollution

APPENDIX C

_ _ _ _ Research No.
1 2 3 4

0 3
_ _ Form No.
5 6

_ Interviewer No.
7

ADVENTIST COLLEGE STUDENT CHROMOSOME STUDY
PERSONAL HEALTH HISTORY FORM

1. **Name** ____
   (Last) (First) (Middle)

2. College Address ____
   (Street or Rm.#) (City) (Zip)

   College Telephone Number ______________

   ☐☐ Date arrived on Campus ______________
   8 9

3. Because we are concerned about chemicals to which you might have been exposed, we would like you to list any full or part-time jobs you have had at any time in the past which might have involved such exposures.

   DO NOT list office jobs, domestic service, or service jobs such as waitress, sales clerk, delivery boy, etc.

   DO list any job in agriculture, manufacturing, industry or service jobs at places like gasoline filling stations, dry cleaners, etc., where you might have been exposed to chemicals.

   IF YOU HAVE NEVER been on a job where you were exposed to chemicals, check this box.

   [ ]

   IF IN DOUBT, PLEASE LIST JOB AND GIVE DETAILS

   Type of Business ____

   Name & Location of Employer ____

   Job Duties ____

   Chemicals ____

   Dates - Mo/Yr FROM ______________ TO ______________

   ☐☐ Approximate Hours Per Week ____
   10 11

   ☐☐☐☐ IF YOU HAVE MORE THAN ONE JOB PLEASE CONTINUE ON NEXT PAGE
   12-15

(continued)

APPENDIX C (continued)

Type of Business ____________________

Name & Location of Employer ____________________

Job Duties ____________________

Chemicals ____________________

	FROM	TO
Dates - Mo/Yr	__________	__________

Approximate Hours Per Week ____________________

IF MORE SPACE IS REQUIRED, PLEASE REQUEST EXTRA PAPER

16 4. Are you allergic to any drugs or medicines that you are aware of?

^{1}[ ] No

☐☐ ^{2}[ ] Yes ⟶ Please list the drugs ____________________

17 18

5. Do you have any diagnosed allergies (for example, asthma, hay fever, asthmatic bronchitis, wheezy bronchitis) or other allergy-related ailments?

^{1}[ ] No

19 ^{2}[ ] Yes ⟶ Please list the environmental substances that you are allergic to (for example, hairs, animal dander, pollen, dust, etc.)

☐☐ ____________________

____

20 21

6. Are you NOW using any food supplements such as Vitamin A, Vitamin C, Vitamin E, or multi-vitamin tablets?

^{1}[ ] No

22 ^{2}[ ] Yes ⟶

Pill	Tablet strength (units, grams, etc.)	Times used per day
______	______	______
______	______	______

☐

23

7. Do you regularly (daily) use aerosol products, for example, hair spray underarm spray deodorant, room deodorizer, etc.?

^{1}[ ] No

24 ^{2}[ ] Yes ⟶ Please list the products ____________________

____

(continued)

8. Do you have any hobbies where you use glue, solvent, paint or chemicals?

25 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please list hobbies and chemicals used.

Hobbies	Chemicals

9. How often do you shave with an electric razor or use a hand hair dryer?

^{1}[ ] Daily

26 ^{2}[ ] 4 or more times per week

^{3}[ ] 3 or fewer times per week

^{4}[ ] Rarely or never

10. On the average, how many hours per week do you watch television?

^{1}[ ] 16 or more hours per week

^{2}[ ] 11-15 hours per week

27 ^{3}[ ] 6-10 hours per week

^{4}[ ] 1-5 hours per week

^{5}[ ] Less than 1 hour per week

11. On the average, how much sunbathing or equivalent exposure to the sun or a sun lamp do you have? (Include work or play in the sun if a considerable part of your body is exposed such as bare back, shorts, bathing suit.)

^{1}[ ] 11 or more hours per week

28 ^{2}[ ] 6-10 hours per week

^{3}[ ] 1-5 hours per week

^{4}[ ] Less than 1 hour per week

12. How frequently do you use cola drinks, including Dr. Pepper, Mountain Dew, etc. (Check nearest category).

^{1}[ ] Almost every day

^{2}[ ] A few times per week

29 ^{3}[ ] A few times per month

^{4}[ ] Rarely

^{5}[ ] Never

(continued)

APPENDIX C (continued)

13. How frequently do you use other caffeine containing beverages such as coffee or tea (except herb type)?

^{1}☐ Daily
^{2}☐ Several times per week
30 ^{3}☐ A few times per week
^{4}☐ A few times per month
^{5}☐ Rarely
^{6}☐ Never

14. How often do you eat meat?

^{1}☐ Daily
^{2}☐ Several times per week
31 ^{3}☐ A few times per week
^{4}☐ A few times per month
^{5}☐ Rarely
^{6}☐ Never

15. What alcoholic beverages have you ever used? Please indicate how often used.

	Frequent	Occasionally	Tried once or twice	Never Used	Date Last Used Mo.	Date Last Used Yr.
^{32} Beer	^{1}☐	^{2}☐	^{3}☐	^{4}☐	______	______
^{33} Wine	☐	☐	☐	☐	______	______
^{34} Hard Liquor	^{1}☐	^{2}☐	^{3}☐	^{4}☐	______	______

35 36

37-40

16. WITHIN THE LAST MONTH have you had any viral infections such as a cold, flu, etc.?

^{1}☐ No
41 ^{2}☐ Yes → Check as many symptoms as you experienced.

- ☐ Sinus/nasal congestion
- ☐ Laryngitis
- ☐ Chest congestion
- ☐ Headache
- ☐ Muscle aches and pains
- ☐ Stomach or intestinal upset
- ☐ Other (describe) ________________________________

(continued)

APPENDIX C (continued)

DURING THE LAST SIX MONTHS HAVE YOU:

17. Had infectious mononucleosis?

42 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give date ____________

18. Suffered from hepatitis (yellow jaundice)?

43 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give date ____________

19. Suffered from hay fever?

44 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give DATE and ____________

list DRUGS taken ____________

20. Suffered from asthma?

45 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give DATE and ____________

list DRUGS taken ____________

21. Had an illness which caused you to break out in a rash?

46 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give DATE and ____________

specify illness ____________

22. Had any illness which prevented you from carrying out your normal activities for more than a week?

47 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give DATE and ____________

specify illness ____________

23. Used or are presently using any prescription medications (including hormones, birth control pills, Darvon, Valium, antibiotics, and other medicines)?

48 ^{1}[ ] None

^{2}[ ] Have used ⟶ Please list medications and give date last taken.

Medication	Date

☐☐
49 50

(continued)

APPENDIX C (continued)

DURING THE LAST SIX MONTHS, HAVE YOU:

24. Used or are using any non-prescription medication such as Aspirin, cough syrup, No-doz, Sleep-eze, etc.?

51 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please list the drugs and give dates last taken.

Medication	Date

☐☐
52 53

25. Received any vaccinations, boosters, or protective serum?

54 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please give dates and list of vaccines or serum (for example, small pox, tetanus, DPT, polio, cholera, yellow fever, typhoid, paratyphoid, gamma globulin).

Vaccine	Date

- - - END OF SIX MONTH SECTION - - -

26. Have you EVER been treated by X-radiation for a medical problem, other than for diagnostic purposes?

55 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please describe medical problem for which therapy was administered and give dates of the therapy.

Medical Problems	Date

27. Have you EVER had diagnostic X-rays other than routine chest and dental X-rays?

56 ^{1}[ ] No

^{2}[ ] Yes ⟶ Please describe medical reason for x-ray, diagnosis, part of body x-rayed, and dates of x-rays if known.

Medical Reason	Diagnosis	Part of body	Date

6

(continued)

28. Have you EVER been given radioactive substances for the diagnosis of a suspected medical condition (such as your thyroid gland)?

57
¹[ ] No
²[ ] Yes ⟶ Please describe suspected medical conditions, test substances, if known, and dates of diagnostic procedures.

Medical condition ____________________

Test substances ____________________

Dates ____________________

29. What medications have you EVER taken routinely for a period of more than one month? (Include purely preventive medications).

58
¹[ ] None
²[ ] Have taken ⟶ Name medication(s) and give inclusive dates of treatment.

Medication	Dates			
	From		To	
	Month	Yr	Month	Yr
________	____	____	____	____
________	____	____	____	____
________	____	____	____	____
________	____	____	____	____

30. How much have you used cigarettes, cigars or pipes?

59
¹[ ] Frequently
²[ ] Occasionally
³[ ] Tried once or twice
⁴[ ] Never used

☐☐☐☐ 60-63
Date last used ____________

31. How frequently have you experimented with

		Frequently	Occasionally	Tried once or twice	Never used	Date last tried
64	Cocaine	¹[ ]	²[ ]	³[ ]	⁴[ ]	______
65	Marijuana	[ ]	[ ]	[ ]	[ ]	______
66	L.S.D.	[ ]	[ ]	[ ]	[ ]	______
67	Other psychadelics	¹[ ]	²[ ]	³[ ]	⁴[ ]	______
68	Amphetamines (Stimulants)	[ ]	[ ]	[ ]	[ ]	______
69	Barbituates (Sleepers)	[ ]	[ ]	[ ]	[ ]	______

☐ ☐☐☐☐
70 71-74

(continued)

APPENDIX C (continued)

32. List the places in which you spent more than two weeks away from home between the close of school last spring and registration at college this fall. Indicate approximate dates and number of weeks spent in each. If you lived in a large city, please give the suburb of the city as well as the city. (It helps if you can list ZIP code as well.)

Dates	Number of Weeks	Town, City or Suburb & City	State	ZIP code
________	________	________________	________	________
________	________	________________	________	________
________	________	________________	________	________

33. Beginning with your permanent address at the time you entered college this fall, please list each place you have lived for six months or more during your lifetime. Work backwards in time. It is not necessary to give street addresses. If you lived in a large city, please give the suburb of the city as well as the city. If you lived in a small town, please give the nearest urban center as well as the town.

PLEASE USE FORM ON FOLLOWING PAGE.

___ ___ ___ ___ ___ ☐
75 76 77 78 79 80
Zip

(continued)

APPENDIX C (continued)

Suburb or Small Town	Nearest Urban Center	State	Zip Code if known	Dates				Check One				
				From		To		Rural Area		Town	Urban Area	
				Month	Year	Month	Year	Farm	Nonfarm		City	Suburb

(continued)

CODING INSTRUCTIONS

Adventist College Student Chromosome Study (SAPS)

Personal Health History Form

Administered September 1976

RECORD 1

Column #	
1- 4	Research Number (I.D. #)
5- 6	Form Number (03)
7	Interviewer Number
8- 9	Date arrived on campus: Number of days on campus before blood drawing.

Days 1-90 = Number of days

Up to:
4 months = 91
5 months = 92
6 months = 93
7 months = 94
8 months = 95
9 months = 96
10 months = 97
11 months = 98
12+ months = 99

If blank, check residence history (p. 9) to see if they live in the area.

10	One type of chemical to which exposed:

1 = Cleaning solvents
2 = Soaps and detergents
3 = Liquid or gaseous fuels
4 = Lacquer/paints/stains
5 = Plastic solvents
6 = Insecticides/herbicides
7 = Other
8 = Unknown
9 = Chemistry lab

(continued)

APPENDIX C (continued)

Column #

11 Length of exposure to chemical:

PART TIME - ≤20 hours/week	FULL TIME - >20 hours/week
1 = ≤3 months	5 = ≤3 months
2 = >3 months to ≤12 months	6 = >3 months to ≤12 months
3 = >1 year to ≤3 years	7 = >1 year to ≤3 years
4 = >3 years	8 = >3 years

12-15 Date of last exposure to chemical: month/year

If no year given, code: month/79

17-18 Drug or medicine allergies:
1 = Antibiotics
2 = Egg white
3 = Horse serum
4 = Other
5 = Unknown

20 Blank

21 Environmental allergies:
1 = Animal
2 = Plant
3 = Inert
4 = Animal & plant
5 = Animal & inert
6 = Plant & inert
7 = Animal, plant & inert

23 Vitamins or food supplements currently using:
1 = Vitamin A
2 = Vitamin B
3 = Vitamin C
4 = Vitamin E
5 = Multi-vitamins
6 = Other
7 = Unknown

35 Second type of chemical to which exposed: (from column 10)

Codes same as column 10

36 Blank

37-40 Date of last use of alcohol: month/year

(continued)

APPENDIX C (continued)

Column #

49-50 Prescription medication currently using:

1 = Birth control pills
2 = Tranquilizers
3 = Stimulants
4 = Antibiotics
5 = Analgesics
6 = Decongestants
7 = Other

52-53 Non-prescription medications currently using:

2 = Tranquilizers
3 = Stimulants
4 = Antibiotics
5 = Analgesics
6 = Decongestants
7 = Other

60-63 Date of last use of tobacco: month/year

70 Number of years of use of drugs

71-74 Date of last use of drugs: month/year

75-79 Zip code of last residence (from p. 9)
If out of U.S., code: 99999

80 Exposure to smog:

GROUP #:	FROM:	TO:
1	low	low
2	low	high
3	high	low
4	high	high

If summer exposure of two weeks (from question 32):

5 = Group 1 with intervening high
6 = Group 2 with intervening high
7 = Group 3 with intervening low
8 = Group 4 with intervening low

(continued)

APPENDIX C (continued)

RECORD 2

Column #

1- 4 I.D.Number

5- 6 Form Number (03)

7 Life-time residence history code:

1 = Low smog history for life-time (x number of years)
2 = Low smog history back x number of years, then ≥ six month exposure to pollution
3 = High smog history for life-time (x number of years)
4 = High smog history back x number of years, then no exposure ≥ six month period.
5 = High smog history back x number of years, then alternating low and high smog history

NOTE: The criteria ≥ six months exposure was used since this was what was asked on the questionnaire in question #33 for life-time residence. If students lived in an area less than that period, they did not necessarily indicate it.

8-10 __ __. __ x number of years (column 7)

11-12 Number of weeks of intervening exposure during the summer as ascertained by question #32.

NOTE: The rule for adding up the number of weeks of intervening exposure was different for each of the groups 5 through 8 as follows:

-for groups 5 and 6 - the total intervening high smog weeks were summed up.

-for groups 7 and 8 - the total intervening low smog weeks were summed.

-Weeks were rounded to the nearest whole week as we did not ask students to list anything less than two weeks. We did not include as intervening early arrival on campus as this information is coded in columns 8 and 9, record 1.

13-14 Number of weeks prior to blood drawing at which the intervening exposure ended. This was taken to be the last intervening high

(continued)

APPENDIX C (continued)

Column #	
13-14 (cont.)	smog exposure for groups 5 and 6 and the last intervening low exposure for groups 7 and 8.
15	Code for intermediate exposure for city: 1 = No exposure to smog 2 = Intermediate exposure 3 = High SO_2 exposure
	Covariate ratings:
16	Chemical exposure (Question #3)
17	Viral infections (Question #16)
18	Mononucleosis (Question #17)
19	Prescription medication (Question #23)
20	Vaccines (Question #25)
21	X-rays or radioactive substances (Questions #26-28)
22	Smoking (Question #30)
23	Drug experimentation (Question #31)

APPENDIX D

___ ___ ___ ___ Research No.
1 2 3 4

0 5 Form No.
5 6

___ Interviewer No.
7

ADVENTIST COLLEGE STUDENT CHROMOSOME STUDY
HEALTH & RESIDENCE HISTORY UPDATE 2 (APRIL, 1977)*

__ __ __ __ __ __ 1. Today's Date ______________________________
8 - 13 (month) (day) (year)

2. If your college residence has changed since your last blood sampling (Nov^mber, 1976), please give your current college address and telephone number.

College Address ____
(Street or Room #) (City) (Zip)

College Telephone Number ____________________________

SINCE YOUR LAST BLOOD SAMPLING (November, 1976) HAVE YOU:

3. Been exposed to chemicals such as cleaning solutions (other than common household cleaning agents), organic solvents, pesticides, glue, paint, etc.

14 ^{1}[ ] No
^{2}[ ] Yes ⟶ Please list:

	CHEMICALS	TOTAL HOURS OF EXPOSURE	APPROXIMATE DATES FROM:	TO:
___ ___ ___ 15- 17	____________	____________	__________	__________
	____________	____________	__________	__________
___ ___ ___ ___ 18 - 21	____________	____________	__________	__________

4. Used any food supplements such as Vitamin A, Vitamin C, Vitamin E, or multi-vitamin tablets?

^{1}[ ] No
^{2}[ ] Yes ⟶

	Medication	Tablet strength (units, grams, etc.)	Times used per day	Date of Last Use
___ 23	____________	____________	__________	__________
	____________	____________	__________	__________
___ ___ ___ ___ 24 - 27	____________	____________	__________	__________

PLEASE CONTINUE WITH QUESTIONS

*HISTORY UPDATE 1, administered in November, 1976, differed only in minor details such as dates so is not included here.

1.

(continued)

APPENDIX D (continued)

SINCE YOUR LAST BLOOD SAMPLING (November, 1976) HAVE YOU:

5. Had any infections or illnesses such as a cold, flu, etc.?

^{1}[ ] No
^{2}[ ] Yes → Please give dates and symptoms for each illness

29 - 32

Illness 1	Illness 2	Illness 3	
Date ________	Date ________	Date ________	
^{1}[ ]	[ ]	[ ]	Sinus/nasal congestion
^{2}[ ]	[ ]	[ ]	Laryngitis
^{3}[ ]	[ ]	[ ]	Chest congestion
^{4}[ ]	[ ]	[ ]	Headache
^{5}[ ]	[ ]	[ ]	Muscle aches and pains
^{6}[ ]	[ ]	[ ]	Stomach or intestinal upset
____________	____________	____________	Other (Please describe)

6. Used or are using any prescription medications (including hormones, birth control pills, Darvon, Valium, antibiotics, and other medicines)?

33
^{1}[ ] No
^{2}[ ] Yes →

Medication	Tablet strength (units, grams, etc.)	Times used per day	Date of Last Use
________	____________	________	________
________	____________	________	________
________	____________	________	________

34 35

36 - 39

7. Used or are using any non-prescription medication such as Aspirin, cough syrup, No-doz, Sleep-eze, etc.?

40
^{1}[ ] No
^{2}[ ] Yes →

Medication	Tablet strength (units, grams, etc.)	Times used per day	Date of Last Use
________	____________	________	________
________	____________	________	________
________	____________	________	________

41 42

43 - 46

8. Received any vaccinations, boosters, or protective serum?

47
^{1}[ ] No
^{2}[ ] Yes → Please give dates and list of vaccines or serum (for example, small pox, tetanus, DPT, polio, cholera, yellow fever, typhoid, paratyphoid, gamma globulin).

Vaccine	Date
____________	________
____________	________
____________	________

(continued)

APPENDIX D (continued)

SINCE YOUR LAST BLOOD SAMPLING (November, 1976) HAVE YOU:

9. Been X-rayed?

48 ^{1}☐ No
^{2}☐ Yes ⟶ Please give

Body Area	Purpose of X-ray
____________	____________
____________	____________

10. Used cigarettes, cigars or pipes?

49 ^{1}☐ Frequently
^{2}☐ Occasionally
^{3}☐ Tried once or twice
^{4}☐ Not used

⟶ Date last used ____________

___ ___ ___ ___ 50 – 53

11. Experimented with:

		Frequently	Occasionally	Tried once or twice	Not used	Date last tried
54	Cocaine	^{1}☐	^{2}☐	^{3}☐	^{4}☐	________
55	Marijuana	☐	☐	☐	☐	________
56	L.S.D.	☐	☐	☐	☐	________
57	Other psychedelics	^{1}☐	^{2}☐	^{3}☐	^{4}☐	________
58	Amphetamines (Stimulants)	☐	☐	☐	☐	________
59	Barbiturates (Sleepers)	☐	☐	☐	☐	________

___ ___ ___ ___ 60 – 63

12. Been away from campus for more than three days?

^{1}☐ No
^{2}☐ Yes ⟶ Please give

___ 64

	Places visited (specify town)	Number of days spent there	Dates
	____________________	__________	__________
___ ___ 65 – 66	____________________	__________	__________
___ ___ 67 – 68	____________________	__________	__________

THANK YOU. PLEASE CHECK TO BE SURE YOU HAVE ANSWERED ALL QUESTIONS.

___ 69

3.

(continued)

APPENDIX D (continued)

CODING INSTRUCTIONS

Adventist College Student Chromosome Study

Health & Residence History Update 2

Administered April 1977

NOTE: Check dates to be sure exposure is only since November 1976

Column #		
1- 4	I.D. Number	
5- 6	Form Number (05)	
7	Interviewer Number - (No one kept track of)	
8-13	Today's date - Month/day/year (Date of blood drawing)	
14	Been exposed to chemicals:	1 = No 2 = Yes
15-16	Two types of chemicals to which exposed: (If exposed to only one type of chemical, Column 16 is left blank)	1 = Cleaning solvents 2 = Soaps and detergents 3 = Liquid or gaseous fuels 4 = Lacquer/paint/stain 5 = Plastic solvents/glues 6 = Insecticides/herbicides 7 = Other 8 = Unknown (unspecified) 9 = Chemistry lab
17	Number of weeks of exposure, for chemical used longest	
18-21	Date of last exposure: month/day	
22	Used any food supplements:	1 = No 2 = Yes

(continued)

Column #

23 Vitamins or food supplements currently using:

(If more than one vitamin is used, code as "5")

1 = Vitamin A
2 = Vitamin B
3 = Vitamin C
4 = Vitamin E
5 = Multi-vitamins
6 = Other
7 = Unknown

24-27 Date of last use of vitamins: month/day

28 Had any infections or illnesses:

1 = No
2 = Yes

29-30 Classify two diseases:

1 = Viral (symptoms 1, 2, 3, 5, 6)
2 = Unknown
3 = Non-viral (symptom 4 only)
4 = Other

31-32 Number of days previous to April blood drawing (from Columns 8-13) for most recent viral disease. Code from last day of illness.

33 Used or are using any prescription medications:

1 = No
2 = Yes

34-35 Prescription medication currently using:

1 = Birth control pills
2 = Tranquilizers
3 = Stimulants
4 = Antibiotics
5 = Analgesics
6 = Decongestants
7 = Other
8 = Unknown (unspecified)

36-39 Date of last use of prescription medicine: month/day

40 Used or are using any non-prescription medication:

1 = No
2 = Yes

(continued)

Column #		
41-42	Non-prescription medication currently using:	2 = Tranquilizers 3 = Stimulants 4 = Antibiotics 5 = Analgesics 6 = Decongestants 7 = Other 8 = Unknown (unspecified)
43-46	Date of last use of non-prescription medicine: month/day	
47	Received any vaccination:	1 = No 2 = Yes
48	Been X-rayed:	1 = No 2 = Yes
49	Frequency of use of cigarettes, cigars or pipes?	
50-53	Date of last use of cigarettes, cigars or pipes: month/day	
54-59	Frequency of use of drugs	
60-63	Date of last use of drugs: month/day	
64	Been away from campus for more than 3 days?	1 = No 2 = Yes, no smog area 3 = Yes, South Coast Air Basin 4 = Not specified
65-66	Number of days in the opposite area (opposite to where the student was going to school when he first joined the study)?	
67-68	Number of days (since last exposure to opposite area) before blood drawing?	
69	Did the student change school since the November blood drawing?	1 = Did not change school 2 = Changed from PUC to LSC 3 = Changed from LSC to PUC 4 = Changed to another college (Not LSC or PUC) 5 = Not in school now

(continued)

APPENDIX D (continued)

Column #	Covariate ratings:
70	Chemical exposure (Question #3)
71	Viral infections (Question #5)
72	Prescription medications (Question #6)
73	Vaccinations (Question #8)
74	X-rays (Question #9)
75	Smoking (Question #10)
76-77	Number of days away in opposing exposure
78-79	Number of days opposing exposure occurs before blood drawing
80	Drug experimentation (Question #12)

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APPENDIX E

_ _ _ _ Research No.
1 2 3 4

_0_ _6_ Form No.
5 6

_ Interviewer No.
7

ADVENTIST COLLEGE STUDENT CHROMOSOME STUDY
HEALTH & RESIDENCE HISTORY UPDATE 3 (FALL, 1977)

_ _ _ _ _ _ 8 - 13 1. Today's Date____________________________
(month) (day) (year)

2. Please give where you can be reached during October.

October Address ____
(Street or Room #) (City) (Zip)

October Telephone Number__________________________

SINCE APRIL 1977 HAVE YOU:

3. Used any food supplements such as Vitamin A, Vitamin C, Vitamin E, or multi-vitamin tablets?

14 1☐ No
2☐ Yes →

Medication	Tablet strength (units, grams, etc.)	Times used per day	Date of Last Use

_ _ 15

_ _ _ _ 16-19

4. Had any infections or illnesses such as a cold, flu, etc.?
1☐ No
2☐ Yes → Please give dates and symptoms for each illness

_ _ _ _ 21-24

Illness 1	Illness 2	Illness 3	
Date______	Date______	Date______	
1☐	☐	☐	Sinus/nasal congestion
2☐	☐	☐	Laryngitis
3☐	☐	☐	Chest congestion
4☐	☐	☐	Headache
5☐	☐	☐	Muscle aches and pains
6☐	☐	☐	Stomach or intestinal upset
______	______	______	Other (Please describe)

PLEASE CONTINUE WITH QUESTIONS ON BACK OF PAGE

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PAGE NUMBER
EPA-287 (Cin)
(8-76)

APPENDIX E (continued)

SINCE APRIL 1977 HAVE YOU:

5. Used or are using any prescription medications (including hormones, birth control pills, Darvon, Valium, antibiotics, and other medicines)? 25

¹[ ] No
²[ ] Yes ⟶

Medication	Tablet strength (units, grams, etc.)	Times used per day	Date of Last Use
______	______	______	______
______	______	______	______
______	______	______	______

__ __ 26 27

__ __ __ __ 28-31

6. Used or are using any non-prescription medication such as Aspirin, cough syrup, No-doz, Sleep-eze, etc.? 32

¹[ ] No
²[ ] Yes ⟶

Medication	Tablet strength (Units, grams, etc.)	Times used per day	Date of Last Use
______	______	______	______
______	______	______	______
______	______	______	______

__ __ 33 34

__ __ __ __ 35-38

7. Received any vaccinations, boosters, or protective serum? 39

¹[ ] No
²[ ] Yes ⟶ Please give dates and list of vaccines or serum (for example, small pox, tetanus, DPT, polio, cholera, yellow fever, typhoid, parathyphoid, gamma globulin).

Vaccine	Date
______	______
______	______
______	______

8. Been X-rayed? 40

¹[ ] No
²[ ] Yes ⟶ Please give

Body Area	Purpose of X-ray
______	______
______	______

9. Used cigarettes, cigars or pipes? 41

¹[ ] Frequently
²[ ] Occasionally
³[ ] Tried once or twice
⁴[ ] Not used

} ⟶ Date last used ______

__ __ __ __ 42-45

(continued)

APPENDIX E (continued)

SINCE APRIL 1977 HAVE YOU:

10. Experimented with

		Frequently	Occasionally	Tried once or twice	Not used	Date last tried
46	Cocaine	^{1}☐	^{2}☐	^{3}☐	^{4}☐	______
47	Marijuana	☐	☐	☐	☐	______
48	L.S.D.	☐	☐	☐	☐	______
49	Other psychadelics	^{1}☐	^{2}☐	^{3}☐	^{4}☐	______
50	Amphetamines (Stimulants)	☐	☐	☐	☐	______
51	Barbituates (Sleepers)	☐	☐	☐	☐	______

___ ___ ___ ___
52-55

11. Please indicate which quarters you were at La Sierra, PUC, not in school, or at another school last year. If you switched campuses during the year or dropped out of school please give dates.

		La Sierra	PUC	Not in school	Attending another school	Date of change
56	Fall '76	^{1}☐	^{2}☐	^{3}☐	^{4}☐	______
57	Winter '77	☐	☐	☐	☐	______
58	Spring '77	☐	☐	☐	☐	______
59	Summer '77	☐	☐	☐	☐	______

___ ___ ___ ___
60-63

12. When was your last blood drawing?

64
^{1}☐ September 1976
^{2}☐ November 1976
^{3}☐ April 1977

13. List the places in which you spent more than two weeks since your LAST blood drawing (as indicated in question 12). Indicate approximate dates and number of weeks spent in each. If you lived in a large city, please give the suburb of the city as well as the city. (It helps if you can list ZIP code as well.)

Dates From	Dates To	Number of Weeks	Town, City or Suburb & City	State	ZIP code
______	______	______	______	______	______
______	______	______	______	______	______
______	______	______	______	______	______
______	______	______	______	______	______

PLEASE CONTINUE WITH QUESTIONS ON BACK OF PAGE

(continued)

14. Because we are concerned about chemicals to which you might have been exposed, we would like you to list any full or part-time jobs you have had at any time since your last blood drawing which might have involved such exposures.

DO NOT list office jobs, domestic service, or service jobs such as waitress, sales clerk, delivery boy, etc.

DO list any job in agriculture, manufacturing, industry or service jobs at places like gasoline filling stations, dry cleaners, etc., where you might have been exposed to chemicals.

IF YOU HAVE NOT been on a job where you were exposed to chemicals, check this box.

[ ]

IF IN DOUBT, PLEASE LIST JOB AND GIVE DETAILS

Type of Business ____________________

Name & Location of Employer ____________________

Job Duties ____________________

Chemicals ____________________

___ ___ ___ 70-72 FROM TO

Dates - Mo/Yr ____________________

___ ___ 73 74 Approximate Hours Per Week ____________________

IF YOU HAVE HAD MORE THAN ONE JOB PLEASE CONTINUE BELOW

Type of Business ____________________

Name & Location of Employer ____________________

Job Duties ____________________

Chemicals ____________________

FROM TO

Dates - Mo/Yr ____________________

Approximate Hours Per Week ____________________

IF MORE SPACE IS REQUIRED, PLEASE REQUEST EXTRA PAPER

(continued)

15. Since your last blood sampling have you been exposed to chemicals such as cleaning solutions off the job (other than common household cleaning agents), organic solvents, pesticides, glue, paint, etc.

¹[ ] No
²[ ] Yes ⟶ Please list:

CHEMICALS	TOTAL HOURS OF EXPOSURE	APPROXIMATE DATES FROM:	TO:
________	________	________	________
________	________	________	________
________	________	________	________

___ ___ ___ 75 - 77

___ ___ 78 79

THANK YOU. PLEASE CHECK TO BE SURE YOU HAVE ANSWERED ALL QUESTIONS.

(continued)

CODING INSTRUCTIONS

Adventist College Student Chromosome Study

Health & Residence History Update 3

Administered September 1977

Column #		
1- 4	I.D. Number	
5- 6	Form Number (06)	
7	Interviewer Number	
8-11	Month/day of blood drawing	
12	Where is the student living now?	1 = La Sierra dorm 2 = La Sierra Village 3 = La Sierra area, not attending LSC (includes those going to school at Loma Linda). 4 = PUC dorm 5 = PUC Village 6 = PUC area, not attending PUC 7 = High area (other than La Sierra area) 8 = Low area (other than PUC area)
13	Year of blood drawing: 6 = 1976 7 = 1977	
14	Used any food supplements	1 = No 2 = Yes

(continued)

APPENDIX E (continued)

Column #		
15	Food supplements - vitamins used.	1 = Vitamin A 2 = Vitamin B 3 = Vitamin C 4 = Vitamin E 5 = Multi-vitamins (or more than one vitamin) 6 = Other 7 = Unknown
16-19	Date of last use of food	
20	Had any infections or illnesses?	1 = No 2 = Yes
21-22	Classify two diseases. (If had only one leave Col. 22 blank, if same disease more than once, take the latest)	1 = Viral (Symptoms 1, 2, 3, 5, or 6) 2 = Unknown 3 = Non-viral (Symptom 4 only) 4 = Other
23-24	Number of weeks previous to Fall 1977 blood drawing (from Columns 8-11) for the most recent viral disease.	
25	Used or are using any prescription medications?	1 = No 2 = Yes
26-27	Classify two prescription medications. (If used only one leave Col. 27 blank)	1 = Birth Control pills 2 = Tranquilizers 3 = Stimulants 4 = Antibiotics 5 = Analgesics 6 = Decongestants 7 = Other 8 = Unknown (or unspecified)
28-31	Date of last use of prescription medication: (month/day)	
32	Used or are using any non-prescription medication?	1 = No 2 = Yes

(continued)

APPENDIX E (continued)

Column #		
33-34	Classify two non-prescription medications used or are using. Same codes as in Columns 26-27.	
35-38	Date of last use of non-prescription medication: (month/day)	
39	Received any vaccinations, boosters, or protective serum?	1 = No 2 = Yes
40	Been X-rayed?	1 = No 2 = Yes
41	Frequency of use of cigarettes, cigars or pipes?	
42-45	Date last used cigarettes, cigars or pipes: (month/day)	
46-51	Frequency of use of drugs?	
52-55	Date last tried drugs: (month/day)	
56	Fall 1976	Indicate where you were.
57	Winter 1977	Indicate where you were.
58	Spring 1977	Indicate where you were.
59	Summer 1977	Indicate where you were. 1 = At La Sierra 2 = At PUC 3 = Not in school 4 = Attending another school (other than LSC or PUC)
60-63	Month/day you switched campuses or dropped out of school.	
64	When was your last blood drawing (previous to this)?	1 = Sept. 1976 2 = Nov. 1976 3 = Apr. 1977
65	Places in which you spent more than two weeks since your last blood drawing?	1 = At the same school (had not been away from school) 2 = Outside South Coast Air Basin 3 = Inside South Coast Air Basin 4 = Both inside and outside South Coast Air Basin 5 = Not specified

(continued)

APPENDIX E (continued)

Column #		
66-67	Number of weeks in opposite area (opposite to place of last blood drawing)?	
68-69	Number of days (since last exposure to opposite area) before blood drawing ?	
70	First chemical exposed to:	1 = Cleaning solvents 2 = Soaps and detergents 3 = Liquids or gaseous fuels 4 = Lacquer/paint/stain 5 = Plastic solvents/glues 6 = Insecticides/herbicides 7 = Other 8 = Unknown (unspecified) 9 = Chemistry lab. 0 = Did not have job with exposure (Only for the 4th drawing. For the 1st drawing, left blank if wasn't exposed).
71	Second chemical exposed to: (If only one chemical Column 71 is blank) (Same coding as Column 70)	
72	Number of weeks of exposure for chemical used longest. (if more than 9 weeks, coded "9")	
73-74	Month of last exposure.	
75-79	Same as 70-74 for off the job exposure.	
80	Where blood was drawn in Fall 1977:	1 = PUC 2 = La Sierra 3 = Loma Linda 4 = Glendale 5 = Other

APPENDIX F

CODING SHEET - LOMA LINDA SMOG STUDY

Column #	
1-5	Student I.D.
6-7	Cells Scored
8	Observer Code
9-13	Date Scanned
14	Sex
15	Record Number
29-30	Abnormal Cells
34-35	Chromatid Breaks
39-40	Isochromatid Breaks
44-45	Hypodiploid
49-50	Hyperdiploid
54-55	Stable Changes

(continued)

APPENDIX F (continued)

Column #	
$\overline{64}-\overline{65}$	Chromatid Gaps
$\overline{69}-\overline{70}$	Isochromatid Gaps
$\overline{71}-\overline{72}$	Triradials, Quadriradials, Polyradials
$\overline{74}-\overline{75}$	Aberrations per Analyzed Metaphase
$\overline{79}-\overline{80}$	Terminal Blebs

(continued)

CODING GUIDE - LOMA LINDA SMOG STUDY

Col.	
1-5	Student Identification Code.
6-7	25 or 75 cells (number of cells actually scored).
8	Observer code (1 = Ina, 2 = Kathy, 3 = Sandy, 4 = Larry and 5 = Irma).
9-13	Dates (9-10 = month, 11-12 = day, 13 = year).
29-30	Abnormal cells include all cells containing breaks, isobreaks, fragments, isofragments, dicentrics, tricentrics, pericentric inversions, rings, isodeletions, and endoreduplications. More than one aberration may occur in a cell. Give number cells, not %.
34-35	Chromatid breaks including fragments and isofragments and isodeletions and 2 breaks each for quadriradials, triradials, pericentric inversions, dicentrics, rings, and four breaks for tricentrics.
39-40	Isochromatid breaks, including isofragments.
44-45	Hypodiploid cells includes 44 and 45 counts.
49-50	Hyperdiploid cells includes 47 counts
54-55	Stable changes include pericentric inversion, dicentric, tricentric, ring, deletion, and translocations.
64-65	Chromatid gaps.
69-70	Isochromatid gaps.
71-72	Triradials, quadriradials, polyradials.

(continued)

Col. Continued

74-75	Aberrations per analyzed metaphase includes the total number of aberrations, including breaks, isobreaks, fragments, isofragments, translocation, pericentric inversions, dicentrics, tricentrics, rings, isodeletions, triradials, quadriradials and endoreduplications, divided by the number of cells scanned (number of aberrations/number of cells). Aberrations are defined as an event such as a break, etc., but an isobreak is counted as one aberration although two breaks have occurred.
79-80	Terminal blebs, including isoterminal blebs which equal two terminal blebs but only one aberration per metaphase.

(continued)

APPENDIX F (continued)

CHROMOSOME ANALYSIS SCAN SHEET

Page ___ Name of Study ________________ Microscope ____________ Date ________________

Culture Time ______________ Patient Number ________________ Sex ________ Age __________

CELL NUMBER

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A-1																		
A-2																		
A-3																		
B																		
C																		
D																		
E																		
F																		
G																		
COUNT																		
COORDINATES																		
PHOTO-X																		

KEY B = Break G = Gap P = Short Arm q = Long Arm C = Centromere

COMMENTS ____

COMPLETED BY ________________ DATE ________________

GLOSSARY

DESCRIPTION OF SCORED CHROMOSOME ABERRATIONS

1. CHROMOSOME - A piece of genetic material which can be clearly seen microscopically at metaphase, or cell division. The major landmarks of a chromosome include the centromere which divides the chromosome into a long arm region and a short arm region. The metaphase chromosome is also seen as two identical halves known as sister chromatids or chromatids.

2. ABNORMAL CELLS - All cells containing breaks, isobreaks, fragments, isofragments, dicentrics, tricentrics, pericentric inversions, rings, isodeletions, and endoreduplications.

3. GAPS AND BREAKS - A gap or break is seen as a separation of part of the chromosome. Two parameters may be used to describe this separation; they are (1) the distance between the pieces and (2) the degree of axial displacement.

   A gap is defined as a small separation (chromatid width or smaller) without axial displacement.

   A break is a larger separation (greater than a chromatid width) or a clear axial displacement.

   Breaks and gaps seen in only one sister chromatid are described simply as breaks or gaps. If both sister chromatids are similarly affected, the term "iso-" is used, e.g. iso-break or iso-gap.

4. CHROMATID AND ISOCHROMATID BREAKS - A discontinuity with displacement in the chromatid arm so that the broken chromatid ends are not aligned. Chromatid breaks includes fragments, isofragments, isodeletions, quadriradials, triradials, pericentric inversions, dicentrics, rings, and tricentrics.

5. CHROMATID GAP - A non-staining and constricted region in the chromatid arm.

ISOCHROMATID GAP - A non-staining and constricted region in both chromatid arms at the same position.

6. ANEUPLOIDY - An incomplete set of chromosomes, that is more than or less than the normal 46 or 23 pairs found in human cells.

   a. HYPODIPLOID - A cell containing less than 46 chromosomes or 23 pairs.

   b. HYPERDIPLOID - A cell containing more than 46 chromosomes or 23 pairs.

7. STABLE CHANGES - Chromosome aberrations likely to be persistent or passed on generation to generation which include pericentric inversions, dicentrics, tricentrics, rings deletions, and translocations.

8. TRIRADIALS, QUADRIRADIALS, POLYRADIALS - End-to-end attachment of chromosomes involving respectively 3, 4, or more chromosomes.

9. TERMINAL BLEBS - An apparently detached end portion of a chromatid separated by less than the chromatid width.

10. DICENTRIC, TRICENTRIC - Aberrations which arise from an exchange between two or more chromosomes which results in the centric products reuniting in such a way to form a dicentric or polycentric structure and an associated acentric fragment.

11. INVERSIONS - Chromosome inversions can be classified into two categories: (a) paracentric inversions where both points of breakage and reunion lie on the same arm of the chromosome; and (b) pericentric inversions where the points of breakage and inversion lie on opposite sides of the centromere.

12. RING - Paired segments of a chromatid with or without a centromere which are joined to give the appearance of a ring.

13. DELETION - Generally paired fragments apparently resulting from a simple break across the chromosome.

14. TRANSLOCATION - Aberrations which involve breakage of two chromosomes and the reciprocal exchange of broken segments between these chromosomes.

15. FRAGMENTS - Pieces of chromatin material characteristically appearing as spheres.